ANNALES MEDICINAE EXPERIMENTALIS ET BIOLOGIAE FENNIAE

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FROM THE DEPARTMENT OF PHARMACOLOGY, UNIVERSITY OF HELSINKI, FINLAND
CHIEF: PROFESSOR A. VARTIAINEN, M.D.

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Turku
Kirjapaino Polytypos
1953

TO MY AUNT ANNI SUHONEN

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M. K. Paasonen.

CONTENTS

I. INTRODUCTION

In animal experiments made in the last years it has been shown that some antihistaminics have the ability to prevent adrenaline pulmonary edema, which is the characteristic reason for death from adrenaline (D'Angostino 1947; Halpern, Hamburger and Cruchaud 1947 and 1948; Vermeil, Halpern and Cruchaud 1949; Halpern, Cruchaud, Vermeil and Roux 1950; Reuse 1948; Schmiterlöw and Wessman 1951; Stone and Loew 1949 b). Partly on the basis of this, Tremonti (1950) treated some patients who had pulmonary edema as a symptom of heart or kidney failure with phenergan. The results were promising in regard to the lung symptoms and Plester and Rummel (1951) mention that antihistaminics are beginning to be used in France as medicines for pulmonary edema. However in preliminary tests carried out on mice it turned out that all antihistaminics did not have this protective effect, but that some of them had a clearly increasing ability on adrenaline toxicity when used in doses that could be considered of therapeutic size. This observation removed the theoretical basis for the use of antihistaminics as medicine in the treatment of pulmonary edema, at least in so far as clinically occurring pulmonary edema and that caused by adrenaline injection are considered comparable to each other. It should be observed that when using antihistaminics in so-called allergic conditions, among which in a restricted sense bronchial asthma is included, adrenaline is often resorted to at the same time. If the adrenaline response is then pathologically strong the consequence can be dangerous because of the antihistaminics used.

In an attempt to explain the phenomenon more accurately the effect of antihistaminics on adrenaline responses was investigated as these can be presumed to play a part in adrenaline toxicity and in the pulmonary edema caused by it especially. In many of their pharmacological properties histamine and adrenaline are an-

tagonistic. Histamine is liberated by adrenaline in physiological conditions (Eichler and Barfuss 1940; Staub, H. 1946 a, b, c; Staub, H. and Baur 1948). On the other hand the injection of histamine effects the increased suprarenal secretion of adrenaline (Dale 1920; Burn and Dale 1926; Mackay 1926). Because it is likely that these substances endeavour to compensate each other's effects a change in reaction is to be expected if the effect of the liberating component, for example of histamine, is prevented by the antihistamines. If the quite opposite effect on the adrenaline response was produced with different antihistamics as in the toxicity tests, it would indicate that specific histamine antagonism has no share in the changing of the adrenaline response. It was of interest to also investigate the effect of antihistaminics on some other typical adrenaline responses as hyperglycemia and mydriasis.

The first antihistamine substance was discovered while seeking new sympatholytic drugs and the known antihistamines are as a rule *in vitro* antagonists to adrenaline. However, depending upon the dose and other considerations, different antihistaminics have either adrenolytic or mimetic properties in the whole animal, in regard to blood pressure amongst other things (see Haas 1951 and 1952; Burn 1950; Leonard and Huttrer 1950).

In this paper the effects of chlor-trimeton and thephorin especially have been compared in different ways. These antihistaminics were chosen because in adrenaline toxicity tests they proved to be the most typical representatives of each group. Chlor-trimeton increased and thephorin decreased the toxicity of adrenaline.

II. MECHANISM OF ACUTE LETHAL EFFECT OF ADRENALINE

In an animal that has died from an injection of adrenaline, congestion is generally found in the lungs at autopsy, and this can be developed into strong haemorrhagic pulmonary edema. At the same time the auricles and ventricles, especially the right, are strongly filled with blood. The bleeding can be in the serous membranes and bloody fluid in the cavities as in the pleural cavity. The effect of adrenaline appears most strongly from an injection directly into the circulation. When given subcutaneously a dose 10 — 50 times compared with the former is needed, due to slow absorbtion (see Hartman and Brownell 1949). As Luisada (1940) puts forward in his survey there are two theories about the lung edema. The cause can be a sudden deficit of the working of the left ventricle or a quick rise of lung capillary permeability caused by nerve reflexes. These theories have also been applied to the lung edema caused by adrenaline. The resulting death of a dog from a large adrenaline dose has generally been held to have been due to the heart (Amberg 1903; Erlanger and Gasser 1919; Freeman *et al.* 1941). If, however, the dose is smaller, on the lethal limit or sublethal, the blood pressure rises and operating through the carotid sinus this causes slowing of the heart rate through vagus action. Breathing is deep and laborious and from the nostrils comes bloody froth, which is the sign of the development of lung edema. Without exception it is held that the death of guinea-pig from adrenaline is caused by lung edema (Elliot 1905; Schmidt 1919). In rabbits and cats also respiration stops before the end of cardiac activity, but in the case of rabbit's death from adrenaline it has been said that this is possible from central respiratory paralysis (Külbs 1905; Erb 1906; Auer and Gates 1917; Halpern, Cruchard,

Vermeil and Roux 1950). In the rat, lung edema is usually the reason for death from adrenaline (Cushny 1909; Eichholz and Hoppe 1933; Riechert and Schmieder 1941; Riechert 1951). The results of Nickerson *et al.* (1950) show that intraperitoneally given adrenaline can cause lung edema in rat, but that death occurs from other reasons already long before a significant amount of edema fluid has accumulated. However, in the rat and in the mouse as well, lung edema is the usual reason for acute adrenaline death (Emmert 1908; Halpern, Cruchaud, Vermeil and Roux 1950; Hoppe *et al.* 1949).

The mechanism of the development of the adrenaline lung edema and lung edema in general is still without final explanation. Toxic adrenaline doses cause cardiac irregularities which may appear as ventricular premature contractions or ventricular fibrillation (Allen 1934). When the blood pressure rises the already damaged heart strains still more and the congestion in the pulmonary circulation gets stronger as the heart insufficiency increases. The situation is formed in which plasma can force its way through the lung capillaries and alveolar epithelium. Adrenaline dilates the lung capillaries and the sympathetic irritation in the lung vessel is nearest the dilatation (see Daly 1933). It must be remembered that, with the rising of the systemic blood pressure, there also follows a rise of pulmonary pressure which is then comparatively much greater, and, on account of the weakness of the walls of the blood vessels, more dangerous. The importance of the different specific reseptories of the cardiovascular apparatus has been explained by the investigations of Luisada and Sarnoff (1946 b). The most important of these is the carotido-pulmonary reflex, whose efferent pathway to the lungs leads along the sympathetic fibres. Whether the reflexes produce first a dilatation of the lung vessel or straightway a rise of permeability is not clear.

Jarish *et al.* (1939 and 1940) produced lung edema by suboccipital injection with veratrine, aconitine, strophantine and cardiazole, which have strong locally irritating characteristics. From this they concluded that the irritation of the rhombencephalic area would cause, besides the moving of the blood to the region of the lungs, local vasomotory changes in the lungs. The part played by the possible central factors in the adrenaline lung

edema has already been investigated earlier. Luisada (1928) and Glass (1928) removed or destroyed certain parts of the brain and their investigations indicate that it is in the quadrigeminal bodies that is situated the area whose destruction weakens the dilatation in the lung vessels caused by adrenaline.

III. HISTAMINE AND ANTIHISTAMINICS

HISTAMINE

It has been found that histamine occurs as a normal component in nearly all animal tissues (Barger and Dale 1911; Abel and Kubota 1919; Best, Dale, Dudley and Thorpe 1927).

Of the pharmacological effects of histamine those concerned with the circulation organs are typical. It causes a fall of blood pressure in the cat, rat, dog, ape and the human being (Dale and Laidlaw 1910 and 1911; Schenk 1921). In the rabbit this happens only under certain narcotic conditions when the constrictive effect of histamine on the lung vessels and bronchioles has been eliminated. The required doses vary greatly: for a dog and a cat 0.01 ~ is already sufficient, but for the rat 20 mg/kg is necessary. Dilatation occurs nearest the periphery, an indication of which already appears for example in a human being as a facial flush after some mikrograms. Histamine's damaging qualities to the capillaries appear when locally induced into the skin as the so called Lewis "triple response" (Lewis, Harris and Grant 1927). Apart from the arteries histamine can also constrict the veins (Franklin 1925) which isolated are even more sensitive than the arteries in this connection (Inchley 1926). Small doses of histamine have no great effect upon the heart (Dale and Laidlaw 1910 and 1911).

Histamine is a substance that causes a change in the smooth muscle tonus, most often constriction (see Feldberg and Schilf 1931). Then almost all smooth muscle organs enter into the question. The uterus of a virgin guinea-pig may already contract in a concentration of 1:250 millions (Trendelenburg and Borgman 1920). The effect on a rat's uterus is nearly paralysing (Vögtlin and Dyer 1925). The intestine of a guinea-pig is particularly sens-

itive: in this even a dilution of 1:1 milliard can cause contraction. The bronchial spasm caused by histamine also appears most clearly in the guinea-pig.

There are many discussions on the physiological significance of histamine. Its appearance in the organism in active and inactive form, its enormous blood vessel activity and quick enzymatic inactivitation are circumstances upon the basis of which it has been regarded as the so called "körpereigene Stoff" and compared to acetylcholine, noradrenaline and adrenaline. Besides the adrenergic and cholinergic nerves the histaminergic nerves have also been spoken about (Ungar 1936).

The most important point in the pathophysiology of histamine is its significance in anaphylactic shock. Both Dale and Laidlaw (1910) and Barger and Dale (1911) put forward the idea that histamine would play an important part in this, because in anaphylactic shock and histamine shock animals react in nearly the same manner. Although there are many circumstances that point to this view, they have been explained by the fact that the histamine liberated in the shock from the organism itself causes a different effect than the histamine coming from outside. Although the part played by histamine in anaphylaxis has been pointed out, it is more difficult to prove that it would be a major factor in producing allergic phenomena in man. It is true that in some allergic conditions, especially in urticaria, the histamine percentage in the blood has risen, but generally no regular changes are found (see Feinberg, Malkiel and Feinberg 1950).

ANTIHISTAMINICS

Because antihistaminics have been supposed to play a part in allergic conditions it is not surprising that attempts have been made to find substances capable of blocking the appearance of histamine reactions in the organism. The number of these whose possible antihistaminic qualities have been investigated is very great (see Feldberg and Schilf 1930). The same applies to the number of drugs whose anti-anaphylactic properties have been investigated (Hill and Martin 1942). Although in many of these substances there are histamine antagonism effects, nowadays they are not regarded as being antihistaminics, because a great speci-

ficness against histamine is demanded from the substances in this group. According to Loew (1947), antihistaminics can be determined as "those drugs which are capable of diminishing or preventing several of the pharmacological effects of histamine and which do so by a mechanism other than the production of pharmacological responses diametrically opposed to those produced by histamine".

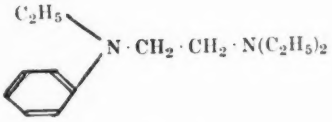
The first antihistaminic that possessed characteristics nearly typical was investigated by the French school under the direction of Fourneau. In the year 1933 Fourneau and Bovet (a, b), and Bovet and Maderni presented the synthesis and qualities of compound 929 F. Staub and Bovet (1937) realised its antianaphylactic effect a little later. In 1939 (a, b) A.-M. Staub demonstrated the antihistaminic effect when she was investigating several synthetic substances of the same type in Fourneau's laboratory. At the same time she observed that 1571 F was a still stronger antihistaminic. The first clinically useful preparation was antergan (2339 RP) which Mosnier synthesised in 1942 in the laboratories of Rhone-Poulenc, and the characteristics of which Halpern first investigated (1942). Neoantergan, which does not differ greatly from the first, proved to be much more effective against histamine shock (Bovet, Horelois and Walthert 1944; Bonvallet and Decourt 1944), and also in clinical use (Decourt 1945). Later the number of synthetic antihistaminics has totalled many thousands, of which only some ten are in clinical use.

In chemical structure the above-mentioned first antihistaminic 1571 F belongs to the ethylenediamine group ($NH_2 \cdot CH_2 \cdot CH_2 \cdot NH_2$). 939 F stems from the ethanolamine group ($HO \cdot CH_2 \cdot CH_2 \cdot NH_2$). Both these substances are in structure typical of two different antihistaminic groups, namely derivatives of ethylenediamine and derivatives of ethanolamine. The greatest part of the known antihistaminics belong to these two groups. Others can be grouped as miscellaneous compounds or as Haas propylamine compounds because in them $CH_3 \cdot CH_2 \cdot CH_2 \cdot NH_2$ is common as a stem. Apart from Haas' there are surveys of many others dealing with the chemical grouping of antihistaminics, such as Hutter's (1948) and Bovet and Bovet-Nitti's (1948). The structure of some common antihistaminics is shown on page 15.

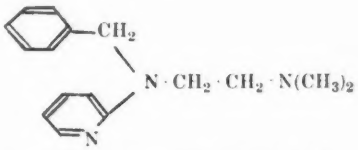
Pharmacology. — In the distribution and metabolism of antihistaminics great differences are not seen. The biggest concentrat-

GRAPHIC FORMULAS OF SOME ANTIHISTAMINE SUBSTANCES

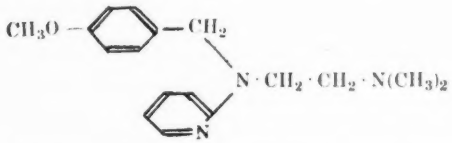
ETHYLENEDIAMINE DERIVATIVES



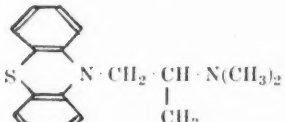
1571 F
N, N-diethyl-N′-phenyl-N′-ethyl-ethylenediamine



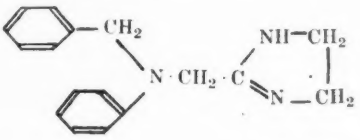
Tripelennamine, "Pyribenzamine"
N, N-dimethyl-N′-benzyl-N′-2-pyridyl-ethylenediamine



Mepyramine, "Neoantergan", "Anthisan"
N, N-dimetyl-N′-p-methoxybenzyl-N′-2-pyridyl-ethylenediamine

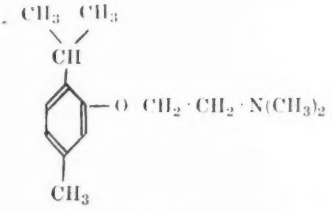


Promethazine, "Phenergan", 3277 RP.
10-(2-N, N-dimethylamino-1-propyl)-phenothiazine

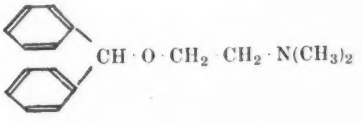


"Antistine"
2-(N-benzylanilinomethyl)-imidazoline

ETHANOLAMINE DERIVATIVES

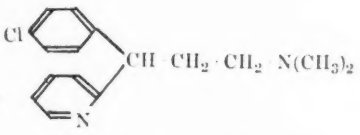


929 F
2-isopropyl-5-methylphenoxy-ethyldiethylamine

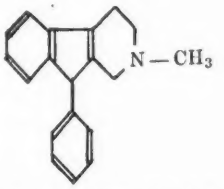


Diphenhydramine, "Benadryl"
β-dimethylaminoethylbenzohydryl-ether

PROPYLAMINE DERIVATIVES OR MISCELLANEOUS COMPOUNDS



Chlor-prophenpyridamine, "Chlor-trimeton"
1-p-chlorophenyl-1-(2-pyridyl)-3-dimethylaminopropane



Phenindamine, "Thephorin"
2-methyl-9-phenyl-2, 3, 4, 9-tetrahydro-1-pyridindene

ion of benadryl after subcutaneous injection was met with in the lungs, spleen and kidneys. The maximum concentration was attained in an hour and in six hours it had disappeared — due to the action of the liver (Clazco *et al.* 1949 a, b, c). The same applies to thephorin amongst others (Dailey *et al.* 1950). Although the histamine sensitivity of the rat and the guinea-pig is very different, their antihistaminic distribution is somewhat similar (Glazco *et al.* 1949 a, b).

The antagonism of histamine can be determined pharmacologically in many ways, of which one is the preventing of bronchoconstriction caused by histamine. Thus a guinea-pig is generally given atomized histamine solution to breathe and the antihistamine dose that clearly prevents spasm is determined. With the newer antihistaminics the result is obtained with smaller doses than before. A.-M. Staub (1939 a) ascertained that 929 F and 1571 F in a dilution of 1:2 millions and 1:10 millions prevented the histamine contraction of isolated guinea-pig ileum. This is also typical of antihistaminics. On the other hand antihistaminics effect with comparatively weaker antagonism the histamine reactions of the cardiovascular system. This applies to whole animal (Bovet, Horclois and Fournel 1944; Reuse 1949 a) and to isolated blood vessels (Dews and Graham 1942; Halpern 1947). Antihistaminics prevent the capillary permeability that has risen with histamine in animals and in humans (Lovejoy, Feinberg and Canterbury 1949). Gastric secretion as well as salivary and pancreatic secretion stimulated by histamine are not inhibited by antihistaminics (Sangster, Grossman and Ivy 1946; Halpern and Duerot 1946).

Antihistaminics have pharmacological effects which can not be considered to have any connection with their histamine antagonism. Their local anesthetic ability may be mentioned. In this respect 1929 F has already been compared with novocaine (Staub, A.-M. 1939 a, b). The most common side reaction of antihistaminics is drowsiness. In the test animals this sedative action appears strongly in those treated with barbiturates, antihistaminics in themselves being only exitants (Winter 1948).

Antihistaminics as drugs acting upon the autonomic nervous system. — In many ways antihistaminics resemble the substances that effect the vegitative nerves. Thus they have variable anti-acetycholine effects, sympathomimetic and sympatholytic properties.

It may be mentioned that the first antihistaminics were discovered when looking for new sympatholytic drugs. Many are spasmolytic drugs. These effects are often only brought out with large doses and the reactions depend much on experimental methods (see Haas 1951 and 1952; Burn 1950; Leonard and Huttrer 1950). Of the experiments in which many different substances are compared may be mentioned Reuse's (1949 a), Schild's (1947), and Haas' (1951 and 1952), where especially the anti-acetycholine effect has been investigated with isolated guinea-pig ileum or guinea-pig seminal vesicle and at the same time compared to the prevention of the effects of histamine and barium chloride among others. Phenergan, benadryl and trimeton possess a rather strong atropine effect. Most of the antihistaminics prevent the falling of blood pressure that follows upon vagus stimulation. In investigations on the guinea-pig seminal vesicle all antihistaminics have adrenolytic abality (Haas 1951). In blood pressure tests the results are often different even though obtained with the same substance. In the isolated carotid artery of cow antihistaminics have a preventing effect both on adrenaline and noradrenaline and synephrine (sympatol), but the doses are more than a hundred times larger in comparison with those that prevent histamine (Buchholz, Hahn and Plester 1951). The different properties probably have significance therapeutically when a non-allergic illness is in question. In many opinions the vegetative nervous system plays an important part in antigen-antibody reactions and sensibility manifestations (Went and Lissak 1935; Heim 1940; Danielopulu 1943, 1944 and 1948 a, b).

IV. DRUGS USED IN THE INVESTIGATION

l-Adrenaline, synthetic — O.Y. Orion, Manufacturing chemists, Helsinki
d, l-Noradrenaline hydrochloride — Ciba Ltd., Basle
Ephedrine hydrochloride — commercial sample
Cocaine hydrochloride — commercial sample
Scopolamine hydrochloride — F. Hoffman — La Roche & Co, Basle
Amytal sodium — commercial sample
Pantocaine hydrochloride — commercial sample
Trypan blue — commercial sample
Trypsine — E. Merck, Darmstadt
Urethane — commercial sample
Antihistamine substances (for structures and chemical formulae see page 15):
"Anthisan", mepyramine maleate — May & Baker Ltd., Dagenham
"Antistine", as methansulphonate — Ciba Ltd., Basle
"Benadryl", Diphenhydramine hydrochloride — Parke, Davis & Co., Hounslow
"Chlor-trimeton", chlorprophenpyridamine maleate — Schering Corp. Bloomfield, N. J.
"Phenergan", promethazine hydrochloride — May & Baker Ltd., Dagenham
"Pyribenzamine", tripelennamine hydrochloride — Ciba Ltd., Basle
"Thephorin", phenindamine hydrogen tartrate — F. Hoffman — La Roche & Co., Basle

For the sake of simplicity l-adrenaline and dl-noradrenaline hydrochloride are referred to in the text as adrenaline and nor-

adrenaline. Fresh solutions of these drugs were made every day and distilled water or saline was used as solvent. Sufficient quantity of hydrochloric acid was used when diluting adrenaline. The amount of all the other above-named substances, which are salts, is always expressed as a salt. If not otherwise stated subcutaneously administered drugs were diluted with distilled water, when injected by other means, the solvent was physiological saline.

I am very grateful to the above-named manufacturing chemists for kindly supplying me with these antihistaminics.

V. LETHAL DOSES OF ANTIHISTAMINICS

Lethal doses of antihistaminics all fall within the same size limits. It appears that a substance that has a low toxicity and slight effect on the central nervous system in the test animals is also clinically the most tolerable (Ercoli *et al.* 1948). When comparing the effect of antihistaminics on the toxicity of adrenaline one must be clear about their own toxicity. Set out below are the lethal doses (LD_{50}) of antihistaminics in mg/kg for mice given subcutaneously in our experiments and the toxicity figures for some other animals when this is of special interest in estimating the poisonousness of chlor-trimeton and thephorin.

Antistine	Mouse, subcut. 200 (Meier and Bucher 1946)
Anthisan	Mouse, subcut. 103 (Winter 1947)
Phenergan	Mouse, subcut. 195 (Winter 1947)
Pyribenzamine	Mouse, subcut. 80 (Ercoli *et al.* 1948)
	Dog, intrav. (25 mg/kg/hour) 49 (Tislow *et al.* 1949)
Benadryl	Mouse, subcut. 125 (Ercoli *et al.* 1948)
	Dog, intrav. (25 mg/kg/hour) 92 (Tislow *et al.* 1949)
Thephorin	Mouse, subcut. and *per os* 270 (Lehman 1948)
	Rat, *per os* 280 (Lehman 1948)
	Guinea-pig, *per os* 185 (Margolin and Tislow 1950)
	Guinea-pig, intraperit. 140 (Lehman 1948)
Chlor-trimeton	Guinea-pig, *per os* 210 (Margolin and Tislow 1950)
	Dog, intraven. (25 mg/kg/hour) 98 (Tislow *et al.* 1949)

Further, giving lethal doses of chlor-trimeton in different ways to mice and rats is said to bear favourable comparison with other antihistaminic drugs in use. (LaBelle and Tislow 1948). In our own experiments when chlor-trimeton was given subcutaneously to mice, its LD_{50} proved to be 200 — 300 mg/kg, as can be seen in Table 9. In our experiments LD_{50} of anthisan to rats after subcutaneously administration was about 150 mg/kg. A lethal dose of chlor-trimeton caused tonic-clonic convulsions in mice, followed by paralysis of the limbs and respiratory failure. There was hyperaemia in the lungs. Generally antihistaminics in toxic doses at first stimulate the test animal, cause respiratory difficulties, convulsions and death after respiratory and cardiac failure. At autopsy congestion is found in the brain, kidneys, liver, and sometimes in the lungs (Ercoli *et al.* 1948; Cruchzit and Fisken 1947).

VI. EFFECT OF ANTIHISTAMINICS ON ADRENALINE RESPONSES

A. TOXICITY OF ADRENALINE

D'Angostino (1947) was the first to establish that the antihistamine substance, antergan, prevents adrenaline from causing pulmonary edema. Halpern and his co-workers (Halpern, Hamburger and Cruchaud 1947 and 1948; Vermeil, Halpern and Cruchaud 1949; Halpern, Cruchaud, Vermeil and Roux 1950) showed that when phenergan was given to rabbits before adrenaline it prevented pulmonary edema resulting in death in all the animals. Anthisan afforded only partial protection. Reuse's (1948) experiments on guinea-pigs supported the theory, but in his test animals there was a noticeable lung change even with phenergan, although much less pronounced. In the same way the work of Schmiterlöw and Wessman (1951), carried out on a much larger number of animals, made it clear that although phenergan exerts a certain protective action against the lethal effect of large doses of adrenaline, there is a marked pulmonary edema in dead guinea-pigs irrespective of whether they have received phenergan or not.

Stone and Loew (1949 a) were unable to discover that anthisan (2.5 mg/kg intravenously) had any preventive effect on adrenaline causing pulmonary edema in guinea-pigs. In the same way (1949 b) they could not establish that phenergan prevented the development of pulmonary edema in rabbits as judged by lung weight, although in large doses (20 mg/kg subcutaneously) it reduced the mortality rate. Winter (1949) likewise was unable to establish that phenergan in guinea-pigs was a protection against a lethal dose of adrenaline, but the dose of adrenaline he used was large, 0.6 mg/kg (mortality ration 6/7 in the control group and 8/8 in those receiving phenergan).

Harpern and his co-workers (Halpern, Hamburger and Cruchaud 1947 and 1948) believe that the preventive characteristic of antihistamine substances on adrenaline pulmonary edema results from the decrease in the cellular permeability. Eichler and Barfuss (1940) and H. Staub (1946 a, b, c) and H. Staub and Baur (1948) have established that adrenaline liberates histamine in physiological conditions. As adrenaline and histamine are antagonistic to each other in many pharmacological effects, it might be supposed that when the level of histamine increases there is a decrease in the toxicity of adrenaline. Schenk (1921) says that a preliminary treatment with histamine blocks the vasoconstrictor effect of adrenaline. In tests with rabbits, injected with histamine before the lethal dose of adrenaline, a clear prevention of pulmonary edema was obtained. (Halpern, Cruchaud, Vermeil and Roux 1950). It is true that Eichler and Barfuss (1940) are of the opinion that histamine is capable of increasing adrenaline sensitivity in cats. The above-mentioned experiments with antihistaminics were carried out using guinea-pigs and rabbits as test animals. Mice and rats are comparatively resistant to histamine (Dale and Richards 1918; Crivellari 1927). It should also be noticed that in mice antihistaminics do not have a preventive effect on histamine toxicity but even increase this. (Bovet and Walthert 1944; Mayer and Brousseau 1946). It is interesting that Halpern and Roux (1949) and Halpern, Cruchaud, Vermeil and Roux (1950) also with mice obtained a reduction in the poisonous effect of adrenaline given intraperitoneally when the animals had first received 20 mg/kg of phenergan. The death rate decreased from 73 % to 53 %. Loew and Micetich (1948 a) gave mice *per os* benadryl and thephorin 50 mg/kg, but the poisonous effect of adrenaline given intraperitoneally did not change.

METHODS

In all the experiments in which the change in the lethal dose of adrenaline was investigated, adrenaline base was diluted with distilled water, using a sufficient quantity of hydrochloric acid. Only when given intravenously was it diluted to saline. Anti-

histaminics were diluted in distilled water 1 : 500 — 1 : 10,000. The antihistamine substance was always injected subcutaneously. 15 — 20 minutes after this adrenaline was injected. When the adrenaline injection was also given subcutaneously, the injection points were as far as possible from one another in the skin of the back. The mice used in the experiments were white adult males. The animals were at room temperature (17 — 19° C.) and were allowed to eat freely during the time of the experiment and before it (rolled oats, oats, white bread, milk and water). The other animals used in the experiments were also allowed to eat. Only those animals which died in the first 24 hours after adrenaline injection, were counted as dead — as later than this death could have been caused by other reasons, such as infection and enteritis (faeces often soft and the animal lost weight). After the adrenaline injection three or four mice were kept in the same glass case.

It is easy to judge the valuation of the lethal doses in respect to the experimental results, directly from the table giving the mortality ratio (number killed / number in group). However, for the sake of clarity the average lethal dose is marked. It is shown graphically from a co-ordinate where the dose scale is a logarithm and the mortality ratio scale a probity one, in so far as there were sufficient observation points. Frequently it is necessary to express LD_{50} in ratio to a certain figure, but it must be emphasised that only roughly comparative results were aimed at in the experiments. Calculation of errors was considered unnecessary.

RESULTS

Mice. — The first tests were made with pyribenzamine and it was discovered that in a dose 4 mg/kg it apparently increased the toxicity of adrenaline. From Table No. 1 it is clear that already the effect of 1 mg/kg seems to indicate potentiation. The survival time was shortest for those given 4 mg/kg pyribenzamine, half of which died in 3 — 5 minutes. Those who were given adrenaline only lasted the longest. Of these all except one lived for sixty minutes or longer.

Table 1

THE EFFECT OF PYRIBENZMINE ON THE TOXICITY OF ADRENALINE GIVEN SUBCUT. (7 MG/KG) TO MICE

Number of Animals	Pyribenzamine mg/kg	Dead	Survival Times after Adrenaline	
			Number of Animals	Minutes
10	—	6	1	15′
			1	60′
			2	90′
			3	>120′
10	1.0	9	3	3 — 7′
			3	20 — 45′
			3	>120′
10	4.0	10	5	3 — 5′
			2	25 — 30′
			2	60′
			1	120′

When anthisan was given before adrenaline the lethal adrenaline dose similarly decreased. This effect appeared with 5 — 10 mg/kg anthisan, but seemed to weaken in large doses (20—50 mg/kg). Although nearly 200 mice were used in these experiments, the result is not tabulated because the animals were not homogeneous and the tests were not made simultaneously.

Because the results of Halpern and his co-workers (Halpern and Roux 1949, Halpern, Cruchaud, Vermeil and Roux 1950) with mice treated with phenergan were contrary, an experiment was performed in which, as they had done, 20 mg/kg phenergan was given subcutaneously and adrenaline intraperitoneally. The result fully corresponded to that of Halpern *et al.*, but again in this experiment anthisan potentiated adrenaline toxicity, as is shown in Table No. 2.

Set out in Table No. 3 are the results of the effect of some other antihistamine substances in general clinical use upon the lethal dose of adrenaline. Chlor-trimeton potentiated adrenaline toxicity most strongly when LD_{50} decreased from about 8 to 3 mg/kg. Benadryl and anthisan have the same effect, although

Table 2

THE EFFECT OF PHENERGAN AND ANTHISAN (20 MG/KG SUBCUT.) ON THE TOXICITY OF ADRENALINE GIVEN INTRAPERITONEALLY TO MICE

Adrenaline mg/kg	Antihistaminics		
	— —	Phenergan	Anthisan
2	3/8 *	0/5	6/7
4	17/20	3/20	16/16
7	10/10	9/10	
LD_{50}	2.3	5.1	<2.0

* Mortality ratio = no. killed/no. in group.

Table 3

THE EFFECT OF ANTISHISTAMINICS (10 MG/KG SUBCUT.) ON THE TOXICITY OF ADRENALINE GIVEN SUBCUTANEOUSLY TO MICE

Adrenaline mg/kg	Antihistaminics						
	— —	Chlor-trimeton	Anthisan	Benadryl	Thephorin	Phenergan	Antistine
2		1/10					
3		4/10	0/6	0/6			
4		8/10	2/11				
5		10/10	4/10	6/10			
6	1/9		7/10	7/9			
7	4/17		6/6	8/8			0/8
8	9/18						
9	6/8					0/6	4/10
11					0/6	1/6	10/12
13					4/15	4/10	10/10
15					4/10	8/10	
18					12/18	8/8	
22					5/6		
LD_{50}	8.0	3.2	5.1	4.5	15.5	13.0	9.5

it does not seem to be as strong. Thephorin has the strongest preventive effect. After this the LD_{50} is 15 — 18 mg/kg. Phenergan also has a rather strong protective effect, but antistine rather a small one. After the three last-mentioned antishistaminics the mortality ratio curves were more shallow than those mentioned earlier. This experiment was carried out with very homogeneous material.

In connection with the poisoning picture, it may be recorded that a few minutes after the injection of adrenaline, the breathing of the mice deepened and became slower. There was blanching of the extremities. Often the back legs and later the front legs as well became paralysed and some cyanosis and exophthalmia and maximum mydriasis appeared. Breathing was then quick and superficial. Sometimes tonic and clonic convulsions occurred, which were clear at least when the animal died quickly. Between the different groups there were no other differences except for the time of death of which those with chlor-trimeton was the shortest, death following in from some minutes to one hour. After thephorin the average was 2 — 4 hours.

At autopsy strong pulmonary edema was almost regularly found. In both lungs there were large hemorrhagic areas, from which at operation flowed bloody fluid. In the trachea there was bloody froth and the lung changes were stronger in those that had died quickly. Both the auricles and the right ventricle of the heart in particular were full of blood. There were no clear differences in the lung weights or other findings of the animals who had died in the different groups.

When adrenaline was injected into the tail vein of mouse nearly the same result was obtained with thephorin and chlor-trimeton as in the earlier experiments (Table No. 4). LD_{50} with adrenaline alone was about 2 mg/kg, which corresponds to the results of Hoppe *et al.* (1949). After chlor-trimeton the dose was about 1 mg/kg and after thephorin 4 mg/kg. The animals died within 2 — 7 minutes with convulsive breathing and usually bloody froth came from the nostrils. The pulmonary edema was stronger than after subcutaneously given adrenaline, but there were no differences in the different groups.

Rats. — As expected the results obtained from rats were the same as from mice. In the test made with 35 two month old rats the lethal dose of subcutaneously given adrenaline was 7 — 8 mg/kg.

Table 4

THE EFFECT OF CHLOR-TRIMETON AND THEPHORIN (10 MG/KG SUBCUT.) ON THE TOXICITY OF ADRENALINE GIVEN INTRAVENOUSLY TO MICE

Adrenaline mg/kg	Antihistaminics		
	———	Chlor-trimeton	Thephorin
0.5		2/6	
1.0	2/11	5/10	0/8
2.0	6/13	8/11	2/8
4.0	8/8		6/12
LD_{50}	2.0	1.0	4.0

Table 5

THE EFFECT OF CHLOR-TRIMETON AND THEPHORIN (10 MG/KG SUBCUT.) ON THE TOXICITY OF ADRENALINE GIVEN INTRAVENOUSLY TO RATS

Adrenaline mg/kg	Antihistaminics		
	———	Chlor-trimeton	Thephorin
0.15	1/6	3/5	
0.25	2/6	4/5	0/3
0.35	3/4	5/5	1/8
0.50			1/4
0.75			3/3
LD_{50}	0.28	<0.15	>0.50
Lung Weight of Dead Animals by % Body Weight	2.5	2.7	2.8

When, fifteen minutes earlier the animals had been given 10 mg/kg anthisan, the average LD_{50} was 4 mg/kg.

Adrenaline was given intravenously to five month old male rats after chlor-trimeton and thephorin (Table No. 5). The results largely resemble those obtained with mice, as has often been ascertained (Hoppe *et al.* 1949). Death followed within 2—15 minutes, seldom

later. The poisoning picture was similar to that found in mice and at autopsy similar changes were found. Also the same phenomenon was observed, that the pulmonary edema was stronger after intravenous adrenaline injection than after subcutaneously. There were no differences in the different groups.

Guinea-pigs. — For female guinea-pigs of 200 — 400 gms. weight, the adrenaline LD_{50} after subcutaneous administration was about 5 mg/kg (Table No. 6). After chlor-trimeton it was about half this and

Table 6

THE EFFECT OF CHLOR-TRIMETOM AND THEPHORIN (10 MG/KG SUBCUT.) ON THE TOXICITY OF ADRENALINE GIVEN SUBCUTANEOYSLY TO GUINEA-PIGS

Adrenaline mg/kg	Antihistaminics		
	— — —	Chlor-trimeton	Thephorin
1.0		0/6	
1.6	0/4	4/8	
2.5	1/3	6/12	0/3
4.0	3/6	4/5	2/9
6.0	5/7		2/7
9.3	3/3		4/8
13.9			5/5
LD_{50}	4.2	2.0	9.3
Lung Weight of Dead Animals by % Body Weight ..... Controls	1.9 1.0 %	2.2	2.0

after thephorin about 9 mg/kg. Thus the reaction is the same as for mice and rats, although the sensitivity of the guinea-pig towards histamine is very great (Leschke 1913), and the animal in question is one that eats different food. At autopsy these animals also had clear pulmonary edema and congestion in the auricles of the heart.

The effect of different doses of chlor-trimeton. — Set out in Table No. 7 are the results of tests in which 1, 10, 50, 100 and 150 mg/kg of chlor-trimeton was given to mice before adrenaline. Even 1 mg/kg

Table 7

DEPENDENCE OF ADRENALINE TOXICITY ON A CHLOR-TRIMETON DOSE IN MICE (BOTH DRUGS GIVEN SUBCUTANEOUSLY)

Chlor-trimeton mg/kg	Adrenaline mg/kg				
	2	3	5	8	LD_{50}
—		0/8	3/14	5/10	8.0
1.0		1/5	3/8	5/5	5.0
10.0	0/8	5/8	9/9		<3.0
50.0	0/4	4/5	5/5		<3.0
100.0	3/5	4/5	5/5		<2.0
150.0	5/5				<2.0

seems to have some effect, but 50 mg/kg does not seem to potentiate more than 10 mg/kg. In larger doses the toxicity of chlor-trimeton itself already comes into the question.

Table 8

ADRENALINE TOXICITY (5 MG/KG SUBCUT.) GIVEN AT DIFFERENT INTERVALS AFTER CHLOR-TRIMETON (10 MG/KG SUBCUT.) INJECTION TO MICE

Time after Chlor-trimeton	Mortality Ratio	Controls without Chlor-trimeton
15 min.	5/5	
1 hr	4/4	
3 „	4/4	0/5
6 „	4/7	0/4

The duration of the effects of chlor-trimeton. — Table No. 8 shows that the potentiating effect of chlor-trimeton on adrenaline toxicity is at least six hours after a subcutaneous injection of 10 mg/kg. There is some evidence that it would then begin to weaken.

Cocaine and adrenaline toxicity. — Cocaine strengthens the toxicity of adrenaline in rats (Riechert and Schmieder 1941). Because Loew and Micetich (1948 a) did not get any change in adrenaline

toxicity by giving mice cocaine (12.5 and 25 mg/kg) *per os*, 10 mg/kg of cocaine hydrochloride (1 : 1,000) was given to 35 mice and twenty minutes later adrenaline subcutaneously. It proved that a 3.2 mg/kg andrenaline dose was regularly lethal to the mice that had been given cocaine, whereas the LD_{50} of the control animals was 7 — 8 mg/kg. The cocaine dose was still not toxic, for the minimum LD for mice is 85 mg/kg when cocaine is given intraperitoneally (Co Tui *et al.* 1943).

Adrenaline and chlor-trimeton toxicity. — Because on the other hand adrenaline increases the toxicity of cocaine (Riechert and Schmieder 1941), experiments were made to see if it has the same effect in regard to chlor-trimeton. Some mice were given 0.5 mg/kg of adrenaline subcutaneously before a toxic dose of chlor-trimeton. From Table No. 9 it can be seen that, at least on the basis of these experiments, one cannot say that the toxicity of chlor-trimeton changes.

Table 9

CHLOR-TRIMETON TOXICITY IN MICE AND THE EFFECT OF ADRENALINE UPON IT (BOTH DRUGS GIVEN SUBCUTANEOUSLY)

Adrenaline mg/kg	Chlor-trimeton mg/kg					
	150	200	250	300	400	LD_{50}
— —	0/4	4/18	4/8	10/14	8/8	250
0.5		3/10	3/6	6/7		$\leqq$250

The effect of chlor-trimeton on adrenaline toxicity after scopolamine and urethane. — Because it has been thought that the central factors are believed to be the cause of adrenaline induced pulmonary edema, the effect of chlor-trimeton was investigated with animals that had been given a large dose of scopolamine hydrobromide or urethane. It is thought that scopolamine particularly depresses the base of the brain (Méhes 1929; Friedberg 1931). Then this drug may have some effect on the area, which Luisada (1928) and Glass (1928) consider to have an important role in the responses of the lung vessels to adrenaline. Urethane is much used as a narcotic in animal experiments. Its effect on the blood circulation and respiration is rather slight. Later it was also often used in blood pressure experiments as a narcotic in this investigation too.

Scopolamine hydrobromide was injected subcutaneously (1:1,000) 100 mg/kg, which is less than one tenth of the LD_{50}. (see Barlow 1932). The other group was given 10 mg/kg of chlor-trimeton at the same time. The lethal adrenaline dose injected 15 — 20 minutes later was 5 — 6 mg/kg for those pretreated with scopolamine only, while for those who had got scopolamine and chlor-trimeton it was 2 — 3 mg/kg.

1.5 g/kg urethane was administered subcutaneously to 24 female rats of 100 — 130 gms. After one hour the other jugular vein was exposed and adrenaline was injected into it in 0.2 — 0.3 ml saline. The lethal dose was on an average 0.5 mg/kg, but for those given 10 mg/kg chlor-trimeton fifteen minutes before adrenaline it was only 0.2 mg/kg. In both tests there was a strong pulmonary edema at autopsy.

DISCUSSION

The first question is, is there a typical sympathomimetic and -lytic action of antihistaminics when they potentiate or decrease adrenaline toxicity. Natural and especially hydrogenated ergot alkaloids raise the lethal dose of adrenaline in the test animals (Rothlin 1925, 1946 and 1947 a; Corelli 1951; Riechert 1951; Halpern, Cruchaud, Vermeil and Roux 1950; Testoni and Moscato 1953). Priscol and to some extent yohimbine administered orally potentiate mice against adrenaline toxicity, but on the other hand 883 F and 933 F have no effect (Loew and Micetich 1948 a). Dibenamine and several other congeners provide market protection (Nickerson and Goodman 1947; Raab and Humphreys 1947; Loew and Micetich 1948 a; Halpern, Cruchaud, Vermeil and Roux 1950). The majority of the drugs known to exert adrenergic blocking activity were found to decrease the toxicity of adrenaline in mice. The mouse screening method is of definite value in selecting and evaluating adrenergic blocking compound, although the reduction of the toxicity in mice does not necessarily indicate adrenergic blocking activity since mecholyl also reduced adrenaline toxicity (Loew and Micetich 1948 a, b). Nickerson *et al.* (1950) especially emphasised that protection afforded against the lethal effect of adrenaline is not a measure of specific adrenergic blocking activity,

because rats after being given adrenaline intraperitoneally primarily died from respiratory failure. This supports the tests in which the lung edema normally produced in rabbits by intracisternal application of irritants, was also able to be prevented with sympatholytics and antihistaminics (Cruchaud and Vermeil 1950 a, b). Stone and Loew (1949 b) believe that "pulmonary edema and death following adrenaline are most effectively reduced by vasodilator drugs which are physiological antagonists of adrenaline, and adrenaline blocking drugs, which pharmacologically block excitatory actions of adrenaline such as vasoconstriction and hypertension", They were unable to show the protecting effect on rabbits with barbiturates, although according to Luisada (1928) hypnotics decrease mortality. 5 mg/kg of atropine was necessary for the mortality of rabbits to lessen to half. This phenomenon brought out with large atropine doses has been ascertained earlier. (Auer and Gates 1917, Bariety and Kohler 1948). In the experiments of Loew and Micetich (1948 a) on mice given, amongst other things, cocaine, atropine (1 and 12.5 mg/kg), pentobarbital sodium (25,50 and 100 mg/kg) and papaverine (25 mg/kg) *per os*, the toxicity of adrenalaine was not changed. That they did not get an effect with antihistaminics may be due to the large doses (50 mg/kg). The increasing effect of anthisan on adrenaline toxicity seemed in our experiments to weaken in large doses. In our experiments with mice, 10 mg/kg cocaine given subcutaneously strengthened adrenaline toxicity.

It is well known that cocaine increases adrenaline toxicity in the same way that it is able to strengthen the typical reactions of small adrenaline doses. (Fröhlich and Loewi 1910; Fischel 1915; Santesson 1919; Eicholz and Hoppe 1933). Thyroxine, which as such, has no effect upon the blood vessels for example, made them sensitive to the action of adrenaline. This sensitivity also appears as increasing adrenaline toxicity (Peltola 1950; Kroneberg and Hüter 1951).

In our recorded experiments antihistaminics divided into two groups, one of which increased the other decreased adrenaline toxicity and particularly pulmonary edema caused by it. This phenomenon was common to rats, mice and guinea-pigs and so cannot have anything to do with the histamine sensitivity of the animals. This is strengthened further by a test after adrenalectomy that follows in the text chapter. Antihistamine potency also cannot

play any part, because for example chlor-trimeton and thephorin are both strong in this respect. Doses of chlor-trimeton all strengthened adrenaline toxicity from 1 mg/kg, but in this regard the maximum effect was already reached with a dose of 10 mg/kg and after the last-mentioned dose it lasted at least six hours. Adrenaline does not increase the toxicity of chlor-trimeton, as it does that of cocaine. Scopolamine and urethane in narcotic doses did not alter the effect of chlor-trimeton, suggesting that there is not any important central site of action. It must be remembered, however, that barbiturates also had no noticeable effect in the experiments of Stone and Loew (1949 b) on adrenaaline toxicity.

If we compare the graphic formulae (see page 15) of antihistamine substances and their effects on adrenaline toxicity it is difficult to find any characteristic difference between both groups. Those of the substances investigated that increase adrenaline toxicity are common to the open ethyleneamine group whose nitrogen is linked with two methyl molecules.

The sympathicolytic or -mimetic characteristics of adrenaline as well as their relation to acetylcholine have been largely investigated in the isolated organs. The results obtained from these experiments cannot support the view that the change in adrenaline toxicity would be a direct consequence of the sympathomimetic or -lytic properties of the antihistaminics in question (see p. 17). To clearly determine the phenomenon, the effect of antihistaminics was investigated especially in blood pressune tests, because the blood pressure rise had been considered one of the important causes of pulmonary edema (Hamilton *et al.* 1938; Stone and Loew 1949 b). Care must be employed in interpreting the results, because among other things dibenamine provides significant protection in fowl, although it does not prevent adrenaline pressor responses (Thompson and Coon 1948). In the most important analysis of pressor responses and pulmonary edema, reactions of the blood vessels and heart appear and these are investigated in their own chapters. Similarly, among others, the question of the permeability changing ability of antihistaminics, which Halpern, Hamburger and Cruchaud (1947 and 1948) regard as the cause of the protecting effect of phenergan. The effect of chlor-trimeton on permeability is investigated later. Further discussion of the subject is put forward together with the later test results.

B. TOXICITY OF NORADRENALINE

In his review (1950) U.S. v. Euler writes that noradrenaline is the chief active principle in the adrenergic nerves and has assumed a central position in autonomic nerve physiology. Since Barger and Dale (1910) it has been known that noradrenaline has the same properties as adrenaline but it has no effect in cases where adrenaline has a blocking effect. It has therefore been suggested by Bacq (1937) that "sympathin E" is noradrenaline. Adrenolytic drugs also block the effect of noradrenaline, but they do not cause "vasomotor reversal" (Barger and Dale 1910; Tainter 1931; Stehle and Ellsworth 1937). It is true that West (1949) states that noradrenaline in large doses after ergotamine and dibenamine may cause a fall in the blood pressure. Cocaine sensitizes the effects of the liberated "sympathin" as does adrenaline (Rosenblueth and Schlossberg 1931) and this same effect has been observed in regard to pure noradrenaline (Tainter 1931). Thyroxine however does not increase the toxicity of noradrenaline as it does that of adrenaline (Kroneberg and Hüter 1951). The ability of noradrenaline to increase the metabolic rate is much weaker than that of adrenaline, as also is its effect in producing hyperglycemia. The effect of noradrenaline on the other hand appears more strongly in the vascular system where it acts as a powerful overall constrictor without noticeably changing the minute volume of the heart. It lacks the vasodilator properties of adrenaline (Euler and Liljestrand 1927; Goldenberg *et al.* 1948). Because adrenaline and noradrenaline differ from each other clearly, at least quantitively, there was reason to investigate whether antihistaminics have the same effect on the toxicity of noradrenaline as of adrenaline.

Antihistaminics such as antergan have only a weak effect on noradrenaline responses (Schmitterlöw 1948).

In the isolated carotid artery of cow the investigated antihistamines changed the noradrenaline reaction in exactly the same way as they did the adrenaline reaction (Buchholz *et al.* 1951).

The toxicity of noradrenaline is less than that of adrenaline, as Biberfeld (1906) found with rabbits and rats and Schültz (1909) with mice. Tainter *et al.* (1948) determined the acute toxicity of intravenously injected l-noradrenaline and l-adrenaline in mice and observed that the latter was about three times more toxic when

the animals were kept in group cages and about eight times more toxic when in single cages. Although the lethal noradrenaline dose in mice causes, especially after delayed deaths, extensive haemorrhagic areas in the lungs and pulmonary edema, it is thought by Hoppe, Seppelin and Lands (1949) that perhaps the cause of death with arterenol may be more complex than with adrenaline.

RESULTS

The method was the same as in the corresponding tests presented earlier. The picture of the poisoning caused by noradrenaline in mice does not differ essentially from that due to adrenaline described earlier. A clear effect came in 5 — 10 minutes. The animals lay quiet, respiration was deep and there was strong exophthalmos. The symptoms were strongest in those given chlor-trimeton, as is also seen from the size of the lethal dose (Table No. 10). The LD_{50} in the controls was about 60 mg/kg, in those given chlor-trimeton about 40 mg/kg and those given thephorin over 100 mg/kg. It seems that the effect of chlor-trimeton in this experiment is weaker than when adrenaline is in question. In the latter the LD_{50} decreased by over 50 %, but in the former by

Table 10

THE EFFECT OF CHLOR-TRIMETON AND THEPHORIN ON THE TOXICITY OF DL-NORADRENALINE HYDROCHLORIDE IN MICE

Noradrenaline subcutaneously mg/kg	Antihistaminics subcut. 10 mg/kg 15 Minutes before Adrenaline		
	—	Chlor-trimeton	Thephorin
15.9	0/5	0/5	
25.2	0/5	1/5	
40.0	1/10	6/15	0/5
63.0	12/22	17/20	1/15
100.0	13/15	18/20	7/15
159.0			5/10
LD_{50}	60.0	40.0	>100.0

hardly 30 %. At autopsy strong pulmonary edema and the typical changes described earlier were found in all the dead animals.

Although there is some doubt as to which is the chief active principle in the adrenergic nerves, adrenaline or noradrenaline, it is interesting to know that the toxicity of both of them increases after an injection of chlor-trimeton and decreases after thephorin. The toxicology of noradrenaline is still little known, but in large doses it seems to be essentially like that of adrenaline.

Chlor-trimeton resembles cocaine in this case also. It differs from the effect of thyroxine but the ability of thyroxine to potentiate adrenaline toxicity is possibly connected with the property of increasing the metabolic rate in both these substances, a property that noradrenaline lacks (Kroneberg and Hüter 1952).

C. TOXICITY OF ADRENALINE AFTER ADRENALECTOMY

On the basis of the investigations of Dale and Laidlaw (1912) and Dale (1920) it is known that adrenalectomy increases the histamine sensitivity of cats. In many later tests this phenomenon has been shown to be common to other animals also (Wyman 1928 and 1929; Gottesman and Perla 1931; Kepinow 1922; Banting and Gairns 1926; Perla and Gottesman 1931; Rose and Browne 1938). Miee are comparatively resistant to histamine. According to Halpern and Wood (1950 a, b) the lethal dose of histamine dihydrochloride injected intraperitoneally is 50 mg/20 g for normal mice. After adrenalectomy the lethal dose was 0.5 mg/20 g. The suprarenal and its cortex especially play an important part against all sorts of noxious agents (see Swingle and Remington 1944 and Sayers 1950). The phenomenon in which the histamine sensitivity of an animal increases has a definite interest when the toxicology of adrenaline is investigated. Based upon the investigations of Staub mentioned earlier, the adrenaline poisoning picture could change with the change in histamine sensitivity. If histamine were a substance used for the contraregulation of adrenaline in the organism, then after adrenalectomy an animal would have the greater possibility of availing itself of histamine and this especially in the case of mice. Although antihistamines do not protect normal mice against histamine (page 23), phenergan or anthisan treat-

ment raises the LD of histamine in adrenalectomised mice to a normal level (Halpern and Wood 1950 b; Halpern and Bourdon 1951). Adrenalectomy also has effect on adrenaline reactions and it is likely that the sensitivity to massive doses becomes greater.

Emmelin and Muren (1949) have determined that antihistaminics injected into the central stump of the coeliac artery cause an output of adrenaline from the suprarenals. This was especially so with the substances that have been found in our investigation to increase the toxicity of adrenaline (anthisan, pyribenzamine, benadryl).

To determine if the suprarenal plays any part in the characteristic change stated earlier on the tolerance of adrenaline in the test-animals given chlor-trimeton and thephorin, an experiment was carried out with mice from which the suprarenals had been extirpated.

METHODS

Bilateral adrenalectomy was performed on 80 male white mice (25 — 30 g) under ether narcosis. The animals were permitted various solid foods, sodium chloride and other salts and water. After operation 0.5 ml of isotonic sodium chloride was injected subcutaneously each day. Within 24 hours of the operation seven mice died and within the two following days a further six. On the fourth and fifth days there were no deaths. It is known that old mice can be kept alive without specific treatment after adrenalectomy (Bomskov and Bahnsen 1935). Adrenaline was injected on the fifth and sixth days and the animals were observed for 24 hours. After this, autopsy was carried out on all of them and the animal was discarded if it was seen that part of the suprarenal was left.

RESULTS

As is clear from Table No. 11, the LD_{50} of adrenaline is from 5 — 6 mg/kg, which is 2 — 3 mg/kg smaller than the LD_{50} of normal animals. After chlor-trimeton it was under 3 mg/kg and after thephorin about 10 mg/kg. These figures correspond in relation to each other with the results presented earlier for intact

Table 11

THE EFFECT OF CHLOR-TRIMETON AND THEPHORIN ON ADRENALINE TOXICITY IN ADRENALECTOMISED MICE. ADRENALECTOMY 5 — 6 DAYS PREVIOUS

Adrenaline subcutaneously mg/kg	Antihistaminics subcut. 10 mg/kg 15 Minutes before Adrenaline		
	—————	Chlor-trimeton	Thephorin
3	0/4	6/9	
5	1/5	8/8	
6	5/7		
7	5/5		2/8
10			4/7
LD_{50}	5 — 6	<3	>7

animals. Those given chlor-trimeton often died within the first hour, whereas those given thephorin after several hours or upon the following day. The life of those given only adrenaline fell between these two times. The poisoning picture differed from that shown earlier in that these animals did not seem to have convulsions before death or they were slight. At autopsy a rather strong pulmonary edema was found in all groups and congestion, especially in the pulmonary circulation.

DISCUSSION

On the basis of this the effect of chlor-trimeton and thephorin cannot occur through the suprarenals. In the findings of Emmelin and Muren (1949) the amount of adrenaline secreted by the suprarenal is very small in comparison with toxic doses. It is interesting that in their experiments adrenaline was liberated by only those antihistaminics which have the — $CH_2 \cdot CH_2 \cdot N \cdot (CH_3)_2$ group in common, linked to nitrogen, as in antergan, anthisan and pyribenzamine or to oxygen, as in benadryl. 929 F and antistine do not have this grouping and they lacked the stated liberating properties of adrenaline. Earlier it has been put forward that antistine did not increase adrenaline toxicity in mice. Phenergan and thephorin decreased adrenaline toxicity in these experiments, and with them also the grouping mentioned above

is not similar. On the other hand in chlor-trimeton it is. In so far as phenergan and thephorin injected into the central stump of the coeliac artery would not cause adrenaline secretion from the suprarenal, but chlor-trimeton would, it might be supposed that this is only one of the characteristic differences between these two groups, but that the suprarenal has no significance in the light of this investigation in the changing of the toxicity after these substances of adrenaline itself.

D. BLOOD PRESSURE

The first demonstrations of diminished pressor response to adrenaline were those of Dale 1905 with ergot alkaloids. The cause of the blood pressure decrease "vasomotor reversal" that then occurs is still unclear. Hydrogenation increases the adrenergic blocking activity of all natural ergot alkaloids too (Rothlin 1946 and 1947 a). β-haloalkylamines, of which dibenamine (N,N-dibenzyl-β-chloroethylamine) may be considered as the prototype (Nickerson and Goodman 1947) also block the blood pressure increase indued by adrenaline. The same applies to benzodioxanes (Haymans and Bouchaert 1935) and yohimbine (Raymond-Hamet 1925). Cocaine, amongst others, increases the adrenaline effect upon blood pressure, and this already appeared in the experiments of Fröhlich and Loewi (1910) on the rabbit and cat and was stated by Santesson (1919) and many others. This is generally explained by the inhibition of adrenaline oxidation. Cocaine and some other local anesthetics inhibit amine oxidase of the rat and guinea-pig liver, cytochrome oxidase and catechol oxidase (Philpot 1940).

The effect of antihistaminics on blood pressure is slight. After a quick intravenous injection temporatory hypotension is usual, and this is generally followed by weak hypertension (Staub, A.-M. 1939 c; Halpern 1942; Sherrod *et al.* 1947). Therapeutic doses have no effect upon blood pressure (McGavack, Elias and Boyd 1946; Feinberg 1946).

Parrot (1943) was the first to realise that antergan given intravenously to cat in a dose of 10 mg/kg strengthened the blood pressure and the nictitating reaction of adrenaline. Halpern

(1942) did not get the same potentiating effect with the same substance. This strengthening characteristic has also been observed in anthisan (Bovet, Horelois and Fournel 1944; Scherrod, Schloemer and Loew 1946; Beauvallet 1949). The effect of benadryl (Loew, MacMillan and Kaiser 1946; Scherrod, Loew and Schloemer 1947; West 1949; Beauvallet 1949; Fabinyi-Szebehely and Szebehely 1952 a) and likewise pyribenzamine (Scherrod, Schloemer and Loew 1946; Yonkman, Chess, Mathieson and Hansen 1946; Scherrod, Loew and Schloemer 1947) also strengthens adrenaline response. Antistine either has no effect (Bock 1950) or "in suitable doses" it increased the adrenaline and noradrenaline response in cats (West 1949), but blocked the pressor response to adrenaline in unanaesthetized rats and guinea-pigs (Löfgren 1949). Phenergan blocks the blood pressure increase produced by adrenaline (Beauvallet 1939; Hazard, Corteggiani and Cornec 1949). It is true that Schmiterlöw and Wessman (1950) did not find this with guinea-pigs. Glanzman and Salvá Miquel (1949) got contrary results in spinal cats, but the phenergan dose used was very small, for example under 50 γ/kg and always under 1 mg/kg. In the chloralose narcosis experiments carried out by Reuse (1949 a) on dogs, 5 — 15 mg/kg benadryl and 5 mg/kg antergan increased the blood pressure effect of adrenaline. 5 — 7 mg/kg of anthisan increased, but 15 mg/kg again decreased it. Antistine 10 mg/kg and phenergan 10 — 12 mg/kg produced a noticeable blocking effect. Lehman *et al.* (1949) pointed out the antiadrenaline effect of thephorin in blood pressure experiments on barbitalized dogs and cats under dial-urethane anesthesia. The percentage inhibition by 1 — 4 mg/kg was 40 — 70. Chlor-trimeton strengthens the pressor effects of adrenaline as is shown by Tislow *et al.* (1949). Trimeton, chemically near to it, also has the same effect (LaBelle and Tislow 1948).

In connection with blood pressure experiments it has been determined that antihistaminics have some effect upon the contraction of the nictitating membrane provoked by adrenaline. This was strengthened by antergan (Parrot 1943), pyribenzamine (Yonkman *et al.* 1946), anthisan and, in suitable doses, benadryl and antistine (West 1949 and 1951 b). On the other hand the antiadrenaline effect appeared with thephorin in this test too (Lehman *et al.* 1949).

Intravenous injections of antihistaminics cause apnea through a reflex mechanism similar to that of veratridine (Aviado Jr., Pontius and Li 1950). The rising of respiratory frequency and amplitude that follows upon this is also typical. On the other hand antihistaminics have the capability of preventing apnea brought on by intravenously induced adrenaline, as Reuse (1949 a) has shown with benadryl and phenergan.

Because just those antihistaminics especially that increased the toxicity of adrenaline seemed to strengthen and those that decreased the toxicity of adrenaline to weaken the blood pressure response, the effects of clor-trimeton and thephorin were compared with the adrenaline reactions of different animals in blood pressure tests. Because especially when conclusions as to the share of blood pressure in adrenaline pulmonary edema are required and also the pressure conditions in the pulmonary circulation are of interest, blood pressure was measured at the same time from the carotid artery, the right ventricle and the left auricle, in some experiments performed on cats. To find out the part played by the splanchnic area, blood pressure tests were also made on eviscerated animals. Oliver and Schäfer in 1895 first considered adrenaline to have a central action. This was supported by many others and Taylor and Page (1951) ascribe on the basis of 127 experiments with dogs the central effect to the action upon chemoreceptors in the brain. Therefore pithed cat was used in some experiments to eliminate the central components. The contraction of the nictitating membrane and respiration was sometimes registered. The effect of adrenaline injected in the femoral or jugular vein and the portal circulation was compared. This procedure had the object of showing if the antihistaminics under investigation had any effect upon the inactivating of adrenaline in the liver. Cocaine strengthens the effect of adrenaline given intraportally more (Dawes 1946; West 1948) than when injected in the femoral vein and prevents *in vitro* liver extract from inactivating adrenaline (Miyake 1952). Yonkman *et al.* (1946) have submitted the possibility that pyribenzamine may dampen certain enzymic systems associated with the degradation of adrenaline or similar agents.

METHODS

The blood pressure was measured with a mercury manometer from the left carotid artery. The test animals were adult males or non-gravid females. The following anesthetics were used: for guinea-pigs 1.5 g/kg urethane intraperitoneally, for rabbits about 1.5 g/kg urethane slowly intravenously, for cats amytal sodium about 70 mg/kg intraperitoneally or 1.5 g/kg urethane subcutaneously. The antihistaminics and adrenaline for guinea-pigs and rabbits were injected into the jugular vein, for cats into the femoral vein with 1 — 3 ml saline. Between two adrenaline doses the interval was at least 5 minutes. For measuring the pressure of the right ventricle a thenylene catheter was pushed along the right jugular vein as is described by Halpern, Cruchaud, Vermeil and Roux (1950). When also the pressure of the left auricle was measured the thorax was opened during artifical respiration and a glass cannula was fastened to the auricle. So as to avoid very large quantities of fluid escaping from the organism when the pressure rose, in these measurements a mercury manometer was also used and not a water manometer. Evisceration was made upon female animals which had been kept without solid food for two days. Then the stomach was removed and also the intestine, spleen, pancreas, omentum and uterus from the abdominal cavity. In the experiments in which adrenaline was injected into the portal circulation, the cannula was generally in the mesenteric vein just before the junction of this and the splenic vein. At least two pressure responses were registered with various doses. Especially when the effect of two antihistaminics upon the same animal was investigated, the same adrenaline dose was injected at least three times after the first antihistamine substance and the response had to be as strong each time before the other antihistaminic injection.

RESULTS

In seven cases out of eight 1 — 3 mg/kg chlor-trimeton increased the adrenaline effect in rabbits. The same was found in the three cases investigated in regard to noradrenaline also. The increase pertaining to the rise of blood pressure reaction and / or its duration was never stronger than 50 %. The adrenaline response after 14

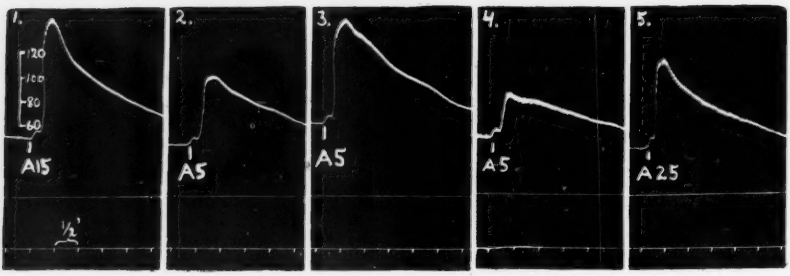


Fig. 1. Spinal cat, 2.8 kg. Blood pressure from carotid artery. Adrenaline (A) γ. Between 2 and 3 Chlor-trimeton 3 mg/kg. Between 3 and 4 Thephorin 2 mg/kg. The response after Chlor-trimeton was repeated three times without any differences

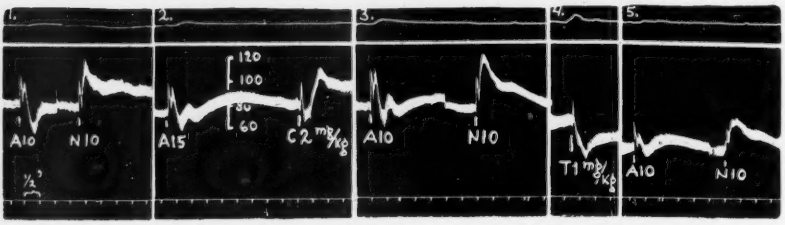


Fig. 2. Cat, 3.7 kg, urethane anesthesia. All records after evisceration. Upper record nictitating membrane, lower carotid pressure. Adrenaline (A) and Noradrenaline (N) in γ. Chlor-trimeton (C) 2 mg/kg and Thephorin (T) 1 mg/kg

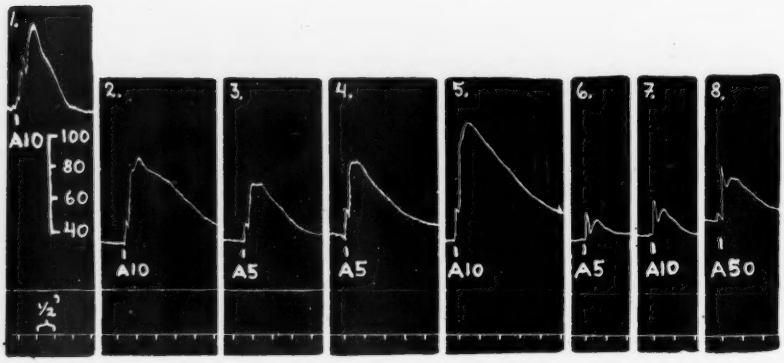


Fig. 3. Rabbit, 2.8 kg, urethane anesthesia. Eviscerationw between 1 and 2. Adrenaline (A) in γ. Between 3 and 4 Chlor-trimeton 2 mg/kg. Between 5 and 6 Thephorin 2 mg/kg

mg/kg chlor-trimeton was only a little stronger than after 2 mg/kg. A dose of 1 mg/kg of thephorin weakened very considerably the blood pressure rise and its duration. Further, after 14 mg/kg chlor-trimeton, 2 mg/kg thephorin brought about a clear decrease. The doses of adrenaline used were 1 — 6 γ and of noradrenaline 5 — 20 γ. 30 γ/kg of adrenaline was given to two rabbits before and after 3 mg/kg chlor-trimeton. The duration of the blood pressure response in the one lengthened from two to six minutes and in the other from 3 to 5.5 minutes, the height being the same. (Because the responses are similar to those presented later in connection with evisceration etc., these are indicated in the figures.) The same result was ascertained in blood pressure experiments in preliminary tests with cats and guinea-pigs also. If adrenaline is followed by a fall of pressure, chlor-trimeton prevents it (Fig. 2). In a few experiments the respiratory embarrassement induced by adrenaline was the same before and after antihistaminics. According to Hertwick *et al.* (1939) barbiturate anesthesia blocks the adrenaline effect of ergotamine and ergotoxine in cats, urethane being the most recommended. In our experiments both these were used without any difference being observed in the effect of chlor-trimeton or thephorin upon adrenaline response as compared with that when amytal anesthesia was used. The "vasomotor reversal" after thephorin was not observed. The effect of antihistaminics when established persists for at least one hour although this question has not been specially investigated. The effect of thephorin appeared clearly even though chlor-trimeton was given prior to it. Lehman *et al.* (1948) showed the same after benadryl.

Spinal Cats. — Typical reactions were obtained from two pithed cats (Dale). Some of the responses of the one cat are illustrated in figure 1. The effect of thephorin on the other cat was not so clear. Decerebration also did not change the noradrenaline response.

Evisceration. — After evisceration blood pressure fell from 120 — 140 to 40 — 70 mmHg. The results for the cat, as also for one of the three rabbits, are shown in figures No. 2 and 3. Chlor-trimeton 2 mg/kg increases the duration and height of the adrenaline response. The effect of thephorin is strong in the same dose, at least for the rabbits. A ten-fold adrenaline dose did not give the same reaction after thephorine as before it. The same results were also obtained with cats given noradrenaline.

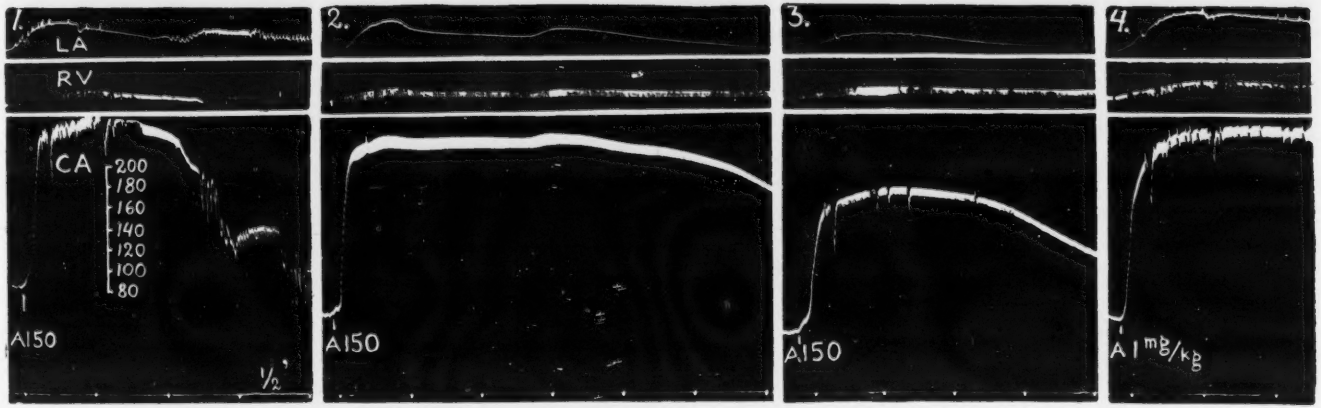


Fig. 4. Cat, 2.6 kg, amytal sodium anesthesia and artificial respiration. Records from upper to below: left auricle, right ventricle and carotid artery. Adrenaline (A) 150 γ, in the fourth A 1 mg/kg. Between 1 and 2 Chlor-trimeton 3 mg/kg. Between 2 and 3 Thephorin 2 mg/kg

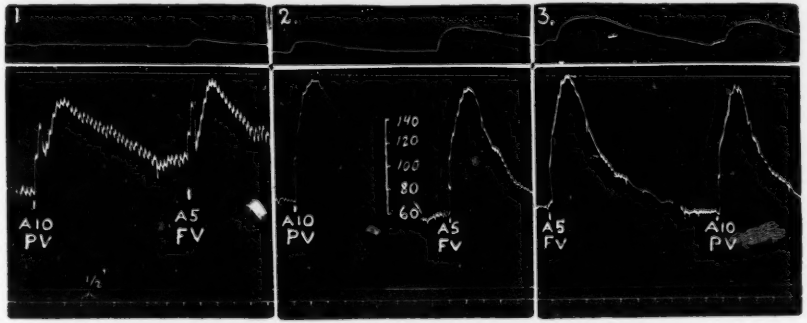


Fig. 5. Cat, 2.2 kg, amytal sodium anesthesia. Upper record nictitating membrane, lower record carotid pressure. Adrenaline in γ into femoral vein (FV) and portal vein (PV). Between 1 and 2 Chlor-trimeton 3 mg/kg and between 2 and 3 Cocaine 1 mg/kg into portal vein

Table 12

THE EFFECT OF CHLOR-TRIMETON (C) AND THEPHORIN (T) UPON THE ADRENALINE BLOOD PRESSURE RESPONSE IN THE CAROTID ARTERY (CA), RIGHT VENTRICLE (RV) AND LEFT AURICLE (LA) IN CATS IN AMYTAL SODIUM ANESTHESIA (ABOUT 70 MG/KG INTRABERITONEALLY). ALL DRUGS WERE INJECTED INTRAVENOUSLY WITH 2 — 3 ML SALINE. SEE TEXT

Body Weight g	Adrenaline γ	Antihist. mg/kg		Pressor Response to Adrenaline						Adrenaline 1 mg/kg	
				before Antih.		after Antih.		Difference %			
				mmHg	Duration	mmHg	Duration	mmHg	Duration	mmHg	Duration
4000	30	C 3.0	RV LA CA	5 3 90	1′	8 3 110	1′	+ 60 ± 0 + 22	± 0	25 4 215	> 12′
3100	30	C 3.0	RV LA CA	0 0 78	35″	0 0 80	50″	± 0 ± 0 + 3	+ 43	26 9 121	2′
2500	30	C 3.0	RV LA CA	6 74	1′10″	9 90	2′10″	+ 50 + 22	+ 86	29 140	2′30″
2400	30	T 2.0	RV LA CA	2 3 122	45″	1 1 38	25″	— 50 — 67 — 69	— 44	12 6 162	3′30″
2300	30	T 2.0	RV LA CA	2 3 64	1′30″	1 2 24	1′25″	— 50 — 33 — 63	— 6	17 4 140	2′55″
2800	30	T 2.0	RV LA CA	7 120	3′	5 32	1′15″	— 29 — 73	— 58	11 87	40″
2600	50	C 3.0	RV LA CA	0 2 131	1′15″	6 3 150	2′10″	+ + 50 + 15	+ 73		
	50	T 2.0	RV LA CA	6 3 150	2′10″	0 4 90	1′45″	— + 33 — 40	— 19	24 13 174	6′45″
2400	150	C 3.0	RV LA CA	32 11 159	1′15″	31 16 164	4′	— 3 + 45 + 3	+220		
	150	T 2.0	RV LA CA	31 16 164	4′	14 7 134	3′10″	— 55 — 56 — 18	— 21	38 3 180	7′30″

Pulmonary pressures. — The eight most successful results from 11 cats are shown in Table No. 12. Adrenaline 30 γ did not have much effect on the pulmonary circulation, although a small pressure rise could generally be noticed. 3 mg/kg of chlor-trimeton mostly strengthened the responses and thephorin (2 mg/kg) weakened them. The effect of thephorin came out clearly. The effect of the antihistaminics was recorded in the same way as the height and duration of adrenaline response. First chlor-trimeton and then thephorin was given to two cats (nos. 7 and 8). Then too the thephorin effect came forth when the reaction before and after was compared. In No. 8 (Fig. 4) it can be seen that 150 γ (about 60 γ/kg) of adrenaline caused a pressure rise in the right ventricle, which was within the same size limits as after a dose of 1 mg/kg. Adrenaline 1 mg/kg was finally given to each cat, when the carotid pressure, espesially that of the right ventricle, also rose. As to the lenght and height, the cat treated with chlor-trimeton had the strongest reaction and the thephorin treated one the reverse. However, on the basis of the material conclusions cannot be formed, because in both groups the reaction differences are great. The beginning of the pressure response was at all three points at the same time or sometimes some seconds before the others in the common carotid artery.

The role of the liver. — Adrenaline was injected into the femoral or jugular vein and portal circulation in eight cats and two rabbits (figs. 5 — 8). Adrenaline doses 1 — 5 γ into the femoral vein corresponded to doses of 4 — 30 γ into the portal circulation, the proportion being on an average 1 : 5. After 3 mg/kg chlor-trimeton through the portal cannula the effect of adrenaline injected in both ways increased in the same proportion. On the other hand the typical weakening was seen after 3 — 5 mg/kg of thephorin. In most experiments 1 mg/kg of cocaine was given in the same way as the antihistaminics and the typical strengthening of the pressure response was clearer after the adrenaline injected through the liver.

Continuous infusion of adrenaline in guinea-pigs. — At the same time as registering the blood pressure and the respiration, adrenaline in saline was given into the jugular vein as a drop infusion with the greatest possible accuracy (0.20 mg/kg/min). The animals had got 1.5 g/kg subcut. urethane. Chlor-trimeton and thephorin was given in 10 mg/kg subcutaneously twenty minutes before the adrenaline infusion was begun. Respiration was registered with a simple

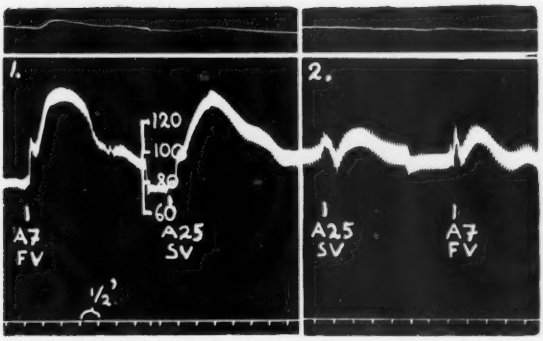


Fig. 6. Cat, 3.5 kg, amytal sodium anesthesia. Upper record nictitating membrane, lower record carotid pressure. Adrenaline in γ into femoral vein (FV) and splenic vein (SV). Between 1 and 2 Thephorin 2 mg/kg into splenic vein

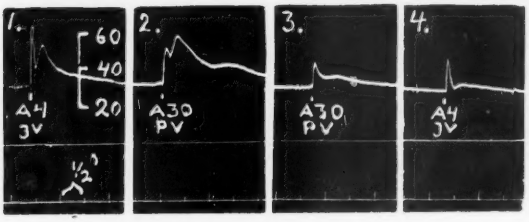


Fig. 7. Rabbit, 3.8 kg, urethane anesthesia. Blood pressure from carotid artery. Adrenaline (A) in γ into femoral vein (FV) and splenic vein (SV). Between 2 and 3 Thephorin 2 mg/kg into splenic vein

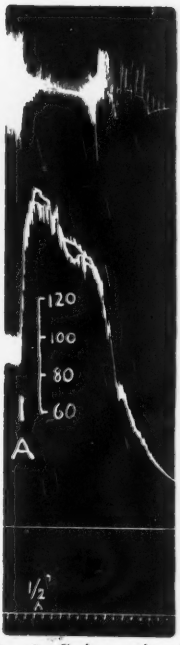


Fig. 9. Guinea-pig, 900 g, urethane anesthesia. Upper record respiration, lower record blood pressure from carotid artery. Adrenaline infusion (0,20 mg/kg/min. into jugular vein) was started by A. 20 minutes before adrenaline Thephorin 10 mg/kg subcutaneously

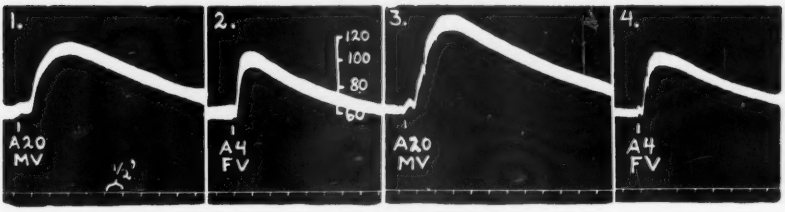


Fig. 8. Rabbit, 4.1 kg, urethane anesthesia. Blood pressure from carotid artery. Adrenaline (A) in γ into femoral vein (FV) and mesenterie vein (MV). Between 2 and 3 Cocaine 1 mg/kg

corrugated rubber tube (pneumograph, Palmer) on the chest of the animal. The results obtained from the ten successful experiments are shown in Table No. 13. The original pressure in the left carotid artery was on an average 75 mmHg and it rose during infusion to the height shown in the table, which is thus the maximum pressure obtained. The duration of the pressure response is the time from the commencement of the infusion to that moment when the blood pressure had fallen to its original value. Apnea that appeared in the respiration at the beginning of the infusion weakened gradually until, at the same time as the pressure fell, there finally occurred respiratory embarrassment, as can be seen in figure 9. On the basis of these results it was impossible to make out any difference between the different groups and because of the great variation of the results further investigations were not made.

Table 13

THE BLOOD PRESSURE RESPONSE AND TOXICITY OF ADRENALINE (0.20 MG/KG/MIN) AFTER CHLOR-TRIMETON AND THEPHORIN (10 MG/KG SUBCUTANEOUSLY 20 MINUTES PREVIOUSLY) IN GUINEA-PIGS UNDER URETHANE ANAETHESIA

Body Weight g	Anti-histaminics	Pressor Response		Lung Weight by % Body Weight
		mmHg	Duration min	
940	—	116	7.0	1.1
900	—	100	7.5	1.3
1070	—	110	12.5	1.2
880	—	140	11.5	1.0
930	Chlor-trimeton	100	8.0	1.3
880	„	106	7.5	1.0
700	„	80	7.0	1.1
1070	Thephorin	110	9.5	1.0
900	„	80	4.5	1.3
700	„	100	11.5	1.5

Nictitating membrane. — The responses of the nictitating membrane in cats under urethane or amytal sodium narcotic may be compared with the corresponding blood pressure reactions (figs. 2, 5, 6). An interesting phenomenon was that thephorin (1 mg/kg or more intravenously) caused the contraction of the membrane, and this corresponded to the reaction from 10 — 20 γ of injected

adrenaline. When thephorin was repeatedly injected this reaction weakened. Further the reaction of intraportally injected adrenaline weakened as compared with this test object. Cocaine increased the adrenaline reaction comparatively more when adrenaline was injected through the portal circulation.

DISCUSSION

The change in blood pressure of the adrenaline response of antihistaminics is difficult to explain for the reason that in other objects of pharmacological investigation their effects are different. In the isolated seminal vesicle of guinea-pig all antihistaminics are sympatholytic (Haas 1952), as when their effect is compared with the isolated carotid-artery of cow (Buchholz *et al.* 1951). The same also applies to noradrenaline. Loew *et al.* (1946) consider that the capability of benadryl to strengthen the effects of adrenaline is most likely due to its atropine properties. In blood pressure tests on dogs benadryl 3 mg/kg had the same effect as 0.03 mg/kg atropine sulphate (benadryl was 1/50 as effective as atropine in antagonizing the action of acetylcholine on intestinal muscle). That there would be some question of a central atropine effect is refuted by establishing that vagotomy does not alter the effect of adrenaline. (Scherrod *et al.* 1947). Although atropine in small doses increases the adrenaline effect on the blood pressure the result is contrary when large doses of atropine enter the question (see Trendelenburg 1929).

In connection with this Reuse's (1949 a) findings are interesting — that anthisan in small doses strengthened the effect of adrenaline and again in large ones blocked it, as the case seemed to be in our experiments concerning adrenaline toxicity. However, this phenomenon does not seem to be characteristic of the other antihistaminics. At least it did not appear with chlor-trimeton or thephorin. In experiments made with isolated organs two antihistaminics, which change the blood pressure response of adrenaline in different ways, are similar to each other in their anti-acetylcholine effects (Haas 1950 and 1952; Schild 1947 b; Reuse 1949 a). Amongst others, benadryl and thephorin have the same value in their relative activity on the isolated intestine (Lehman 1948). Spasmolytic activity is also common to antihistaminics (see Haas 1951 and 1952). Actual

antihistaminic characteristics probably cannot play any part, because chlor-trimeton and thephorin for example are both strong in this respect. The liberation of histamine cannot under these circumstances play any part.

It has been shown that some of the antihistaminics investigated have anti-nicotine effects on blood pressure (Reuse 1949 a; Hazard *et al.* 1949). However, it is not known if the possible ganglion effect of antihistaminics could change adrenaline response. Tetraethylammonium salts sensitize the pressor responses to adrenaline and noradrenaline (Clair and Stone 1951). The site of action of TEA is believed to be in the adrenals (Hugues and Lecomte 1951).

It is difficult to determine from these experiments what part the heart played in blood pressure response, but the effect could not be due alone to the increase of minute volume caused by the frequency increase of the heart. In fig. 4 the quinidine effect of chlor-trimeton appears, for strong premature contractions are lacking after 3 mg/kg chlor-trimeton. This chinidine effect can not have any share in the strengthening of the blood pressure response, at least in this case. Thephorin also has similar characteristics (see page 55).

It has been thought the vasodilator centres situated in the brain and medulla might play some part in adrenaline response (Trendelenburg 1929). Experiments with spinal cats in which the results were the same as for the intact animal, remove the share of the possible central reflexes and similarly of the central vagus excitation.

Because the maximum constriction occurs in the splanchnic vessels it might have been expected that the investigated phenomenon would have appeared at least weakly after evisceration. However, at evisceration the blood pressure fell strongly but the adrenaline response was the same as before (fig. 3). The explanation of this may be that the constrictor effect of adrenaline is the stronger the lower the tonus. On the basis of this experiment it cannot yet be said that the typical effect of chlor-trimeton and thephorin on blood pressure reaction would appear in the skeletal muscle vessels besides the visceral vessels, because the liver, kidneys and lungs were not removed. In the experiments in which the effect of adrenaline injection was measured in the pulmonary circulation the intention was to investigate as far as possible if antihistaminics could possibly

have any specific effect, especially upon the reactions of this area. Because the pressure rise in the pulmonary blood vessels may be an important factor in the pathogenesis of adrenaline pulmonary edema it might have been expected that chlor-trimeton would strengthen the pressure just there and thephorin have the opposite effect. However the results do not point to any specific change in blood pressure response appearing, but that, in so far as there is any change, it follows the same alterations as the pressure of the systemic blood circulation. Both in the right ventricle and in the left auricle the response change after antihistamine was about the same, so that no suggestion of change in pulmonary constriction or of changed reaction in the other side of the heart was obtained. Also in the work of Halpern, Cruchaud, Vermeil and Roux (1950) on rabbits, the blood pressure being measured in the right ventricle, there was no difference between those that had been given phenergan and the control animals.

In the experiments demonstrating the part played by the liver in the change of adrenaline effect of antihistaminics the requisite adrenaline dose proved to be about five times greater when injected through the liver, so that the same response would have been obtained as when given into the femoral vein. In smaller doses the difference was more apparent than in big ones. This corresponds to the results of Philpot and Cantoni (1941) and Dawes (1946) among others. On the basis of our experiments the antihistaminics investigated cannot be presumed to have any effect on the amine oxidase that decomposes the adrenaline in the liver or upon other adrenaline decomposing enzymic systems as was suggested by Yonkman *et al.* (1946). Only in one test did chlor-trimeton relatively strengthen further the effect of adrenaline given into the portal circulation, but then the cannulla was tied to a small mesenteric vein more peripheral than usual. The fact that antihistaminics constrict the blood vessels in high concentrations, as is mentioned later may be explained by the adrenaline amount injected reaching the general circulation in a higher concentration. Cocaine (1 mg/kg intraportally) strengthened noticeably the effect of adrenaline when given through the portal cannulla. It may be mentioned that ephedrine (Blaschko *et al.* 1937) and methylene blue (Philpot and Cantoni 1941) also, which inhibit the action of amine oxidase *in vitro* and *in vivo* potentiate the pressor effect of

adrenaline injected into the portal circulation of spinal cat (Philpot and Cantoni 1941; West 1951 a, b).

It is difficult to explain why chlor-trimeton by using continuous infusion on guinea-pigs did not change the adrenaline reaction any more than thephorin did. It has not been explained if death was primarily caused by pulmonary lung edema, which was relatively rather weak, by central respiratory failure or by paralysis of the heart. Because the degree of pulmonary edema was so slight that it was insufficient to explain death this could be put down to respiratory failure or cardiac failure. As it has been shown that these antihistaminics have effect on adrenaline pulmonary edema no action can be expected here. According to Massons *et al.* (1945) "collapse from adrenaline seems to be due to the duration of hypertension and ischemia rather than to the absolute amount of adrenaline injected". For example the same dose injected into dogs over one hour caused an irreversible collapse, but given once the collapse was reversible. Because the dose in our experiment was rather large the result would possibly have been the same even though the dose had been varied to some extent to one side or the other.

Because the nictitating membrane reaction was the same as that of the blood pressure it would be interesting to know if its reaction would also be the same when isolated. If both these antihistaminics were then sympatholytic, it might be supposed that the effect upon the seminal vesicle of a guinea-pig would be different *in vivo* than *in vitro*. Has thephorin parasympathetic stimulating properties (Rosenblueth 1932), because it has the ability to contract the nictitating membrane?

E. ISOLATED HEART

Besides vasoconstriction the increase of minute volume caused by cardiac stimulation plays a part in the blood pressure rise after adrenaline. It is known that adrenaline usually increases the frequency and amplitude of the heart. Although adrenaline prevents the establishment of ventricular fibrillation by faradization and promotes the recovery of the heart muscle from fibrillation (Smith and Mulder 1936), it has the ability to effect the heart muscle so that ventricular rhythms appear. Ergotoxine and other

ergot alkaloids are mutually antagonistic to the adrenaline stimulation of the frog heart (Rothlin 1925; Langecker 1925). Quinidine, ergotamine (Allen *et al.* 1941) and procaine (Burstein and Marangoni 1940) prevent ventricular fibrillation produced by cyclopropane and adrenaline. On the other hand cocaine prolongs the cardiac responses to adrenaline (Peralta and Lizarralde 1946). Many workers have reported the failure of adrenergic blocking agents to prevent stimulation of the mammalian heart muscle by adrenaline (see Nickerson 1949).

The ability of antihistaminics to change the adrenaline effect on the heart has not been widely investigated. On the other hand it has often been ascertained that antihistaminics prevent ventricular fibrillation when adrenaline is given to dogs sensitized with chloroform. This quinidine effect has been shown with anthisan (Dews and Graham 1946), pyribenzamine and antistine (Levitan and Scott 1949) and benadryl (White and McCawley 1950). Schallek (1952) ascertained with dogs under pentobarbital narcosis that thephorin blocked adrenaline-induced premature ventricular contractions more strongly than quinidine. It has also been shown that antihistaminics have toxic effects that bear directly upon the heart (see Haas 1951 and 1952), and these appear as contractility and frequency disturbances or decreasing of muscle power. Orias, Gilbert and Brooks (1949) gave to cats under dial narcosis, antistine, benadryl, pyribenzamine, thenylene and thephorin. The 4 and 8 mg/kg doses produced auriculoventricular block, intraventricular block, slowing of the rate and changes in the electrical axis. The effects were well correlated with the dose. Death after lethal doses was due either to respiratory paralysis and/or cardiac failure. Bunse and Hahn (1950) determined ECG changes with all antihistamines derived from ethylenediamine.

The purpose of this investigation was to find out if differences from the normal in adrenaline response could be shown with the isolated heart, by using a small concentration of chlor-trimeton or thephorin in perfusion fluid.

METHODS AND RESULTS

Frog heart. — In May-June, tests were made with the isolated frog heart (Rana Temporaria) according to Straub's method.

The frogs had been collected in the November of last year, from which time they were kept without food at a temperature of + 5 — + 8° C. Some days before the test the animals were brought into room temperature (+ 18 — 19° C.). The perfusion fluid in which the investigated solutions were made was oxygenated isotonic Ringer solution. A perfusion pressure of about 5 cms of water was employed. The antihistaminics were allowed to have effect for about 2 minutes, after which the Ringer solution was changed, in which there was the same amount of antihistamine drug and also the adrenaline concentration under investigation.

In a concentration of 1×10^{-5} chlor-trimeton caused a negative inotropic effect, which in a concentration of 1×10^{-4} appeared in a few minutes in systolic contracture (fig. 10). The effect with thephorin was still clearer (fig. 11). The effect was reversible and the heart that had already ceased beating was always revived with a change of Ringer.

The positive inotropic and/or chronotropic effect caused by adrenaline did not change when using antihistamine substance concentrations, ineffective as such, of 1×10^{-7} to 1×10^{-8}. Similarly the effect of chlor-trimeton of 0.5×10^{-5} did not change. In Ringer that was poor in calcium or potassium rendering the heart hypotonic did not effect the result.

Rabbit heart. — Isolated rabbit hearts were perfused with 36 — 37° C. of well oxygenated Ringer's solution according to Langendorff's instructions. The pressure was kept stable by means of a Boyle-Mariotte flask. The heart beat was registered throughout the test and the volume of coronary flow was measured every minute for five minutes before and after adrenaline injections. Adrenaline usually 0.1 — 1.0 γ, was injected with a little volume of Ringer's solution into the perfusion solution above the heart and it was estimated to dilute to 6 — 8 ml. The reaction of each heart was investigated at the beginning in pure Ringer solution, then in Ringer solution containing 2×10^{-6} chlor-trimeton and finally the same amount of thephorin.

In the above-mentioned concentration chlor-trimeton did not noticeably change the heart beats registered. On the other hand thephorin in all experiments decreased the pulse amplitude. The same happened in one experiment in which it was in a concentration of 1×10^{-6} (fig. 12).

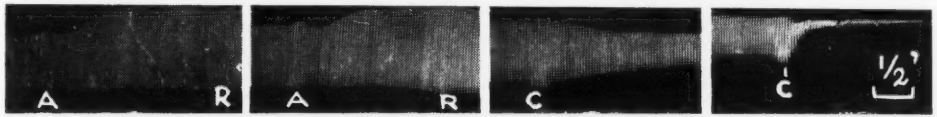


Fig. 10. Frog heart (Straub). 1: Adrenaline (A) 1×10^{-8}; 2: A 1×10^{-8} in Chlor-trimeton (C) 0.5×10^{-5}; 3: C 1×10^{-5} and 4: C 1×10^{-4}.

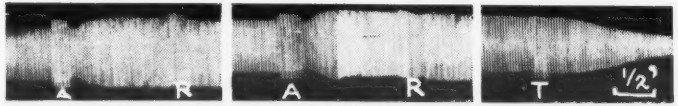


Fig. 11. Frog heart (Straub) 1: Adrenaline (A) 1×10^{-6}; 2: A 1×10^{-6} in Thephorin (T) 1×10^{-6} and 3: T 1×10^{-5}

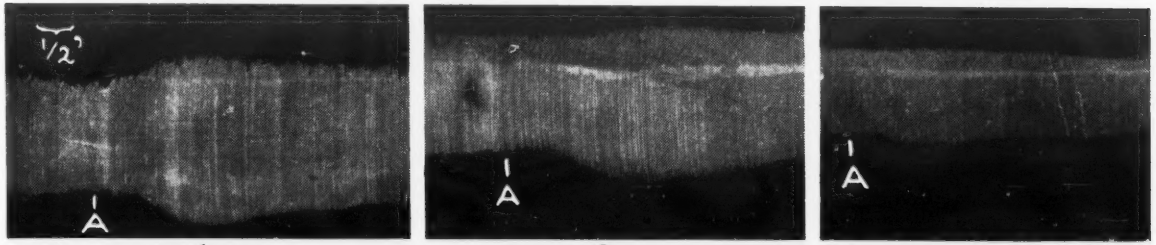


Fig. 12. Rabbit heart (Langendorff). 1 γ Adrenaline (A) was injected into perfusion fluid. 2: Ringer with Chlor-trimeton 2×10^{-6} and 3: Ringer with Thephorin 2×10^{-6}

Chlor-trimeton did not noticeably change the positive inotropic and chronotropic effect of adrenaline. The effect of adrenaline in thephorin Ringer solution did not appear in normal strength (fig. 12). However this does not prove anything, because the heart contractions had already weakened in the manner described earlier, because of the pure antihistaminic. On the other hand it is a well-ascertained fact that the hypotonic heart reacts more powerfully to adrenaline than normally and for that reason a strengthening of the adrenaline effect might also have been expected. The coronary inflow (ml/min) did not increase at all after adrenaline but during the first two or three minutes it decreased from 10 — 30 %. This is typical of a small dose of adrenaline, as is clear from the work of Melville and Lu (1950). Chlor-trimeton and thephorin did not change the adrenaline effect in this respect either.

DISCUSSION

On the basis of the tests presented it cannot be supposed that antihistaminics would change the blood pressure reaction of adrenaline by sensitizing or desensitizing the heart. However it must be taken into account that adrenaline bradycardia for example is a consequence of the reflectorically risen blood pressure (Haymans 1929) and that adrenaline effects the heart also through the action on the centres in the medulla (Brown 1916). Then the central vagus stimulation is an important cause of different rhythm disturbances. With the isolated heart these possibilities do not exist. However, as is mentioned before, decerebration did not change the typical effect of antihistaminics on adrenaline responses. In the tests a concentration of 2×10^{-6} of thephorin already paralyses the heart of rabbit, but much larger concentrations *in vivo* are ineffective in this respect. To block the heart effects of histamine greater antihistamine doses are needed than in other tests respectively. As has been said earlier, the case is the same with the adrenergic blocking agents and the adrenaline in this test object. There is some similarity in this respect between antihistaminics and adrenolytics as in their effects upon the peripheral circulation.

F. PERIPHERAL CIRCULATION

After adrenaline injection the blood pressure increase that follows is mainly caused by strong vasoconstriction. The effect is directed to the capillaries as well as the arteries, as appears in the isolated organ (Hooker 1920). Although adrenaline, in small doses especially, may cause some dilatation also, the constriction of the isolated organ vessels in perfusion is the most usual reaction, as is seen in the hind legs of the frog (Laewen-Trendelenburg) and in the rabbit ear (Pisemsky). As is known ergot alkaloids have the ability to prevent the vasoconstrictor effects of adrenaline in the isolated organ. Many other specific inhibitors of adrenaline, such as dibenamine, yohimbine and regitine, also have this ability. Burn and Dutta (1948) also noticed that benadryl had this property, as did anthisan. In Fleckenstein's experiment (1952) adrenolysis was clearly brought out in the ear of a rabbit with some other antihistamines.

In the following the effect of chlor-trimeton and thephorin on the reaction caused by adrenaline in the peripheral blood vessels, is investigated.

METHODS

In the experiment rat hind leg preparation was used, as is described by Burn (1952). The hydrostatic pressure was 20 — 30 cm water and the dropping speed was measured using a Condon drop counter (Palmer) or Inchley's drop recorder (Palmer). As perfusion fluid Ringer solution with glucose was used and in this the solutions of chlor-trimeton and thephorin were made as strong as 1×10^{-6}, 1×10^{-5}, or 1×10^{-4}. Before the adrenaline injection there was perfusion with antihistamine for four minutes or longer. Injections of test doses of adrenaline (0.1 — 0.2 ml) were made into a small rubber topped chamber, through which the perfusion fluid passed before entering the cannula. The fluid capacity to which the injected adrenaline was diluted was about 2.5 ml.

RESULTS

Chlor-trimeton alone slowed the dropping speed when its concentration in perfusion fluid was 1×10^{-4} (Fig. 13). In a concen-

tration of 1×10^{-5} it no longer had effect. In a concentration of 1×10^{-4} pure thephorin had no effect, but 0.5×10^{-3} quickly produced the maximum vasoconstriction (Fig. 14).

Adrenaline caused vasoconstriction always, which was apparent in the concentrations 0.8×10^{-7} — 0.4×10^{-6}. The reaction in the same preparation did not change much in so far as the dropping speed had not slowed greatly. Each time the effect of the comparable adrenaline dose was investigated at least twice. When 1×10^{-6} of chlor-trimeton was in the perfusion fluid, two to four times the adrenaline dose was required in comparison with the norm. In the concentration of 1×10^{-5} the dose had to be three to seven times for the effect to be the same as before antihistamine. Thephorin reduced the constrictor action of adrenaline in the concentration of 1×10^{-6} to one seventh to one tenth. In larger concentrations the effect was still stronger. In a concentration of 1×10^{-5} often adrenaline, even at a strength of 0.8×10^{-4}, was necessary and even then the effect did not appear especially strongly.

In some tests the isolated rat lung was perfused with 37° C. Ringer solution through a cannula that had been put into the pulmonary artery. The adrenaline effect did not appear or it was very indefinite.

DISCUSSION

In all the concentrations tested, thephorin and chlor-trimeton had antiadrenaline potency and this was the stronger the stronger the concentration. In this respect thephorin is the stronger. This is in conformity with the experiments of Fleckenstein (1952), in which the molar concentration required to reduce the constrictor action of adrenaline to a tenth was M/640,000 for thephorin and the same for phenergan. The other antihistaminics were weaker adrenolytics in the following order: antistine (M/128,000), pyribenzamine (M/64,000), anthisan (M/32,000) and benadryl (M/16,000). A concentration of 1×10^{-5} of chlor-trimeton is M/27,500, so that it must be regarded as equal to benadryl or weaker than it in the proportions investigated, because the adrenolysis was not so strong as mentioned earlier. A concentration of 1×10^{-6} of thephorin in which the blocking was within the same size limits as in Fleckenstein's experiment, corresponds to M/410,000.

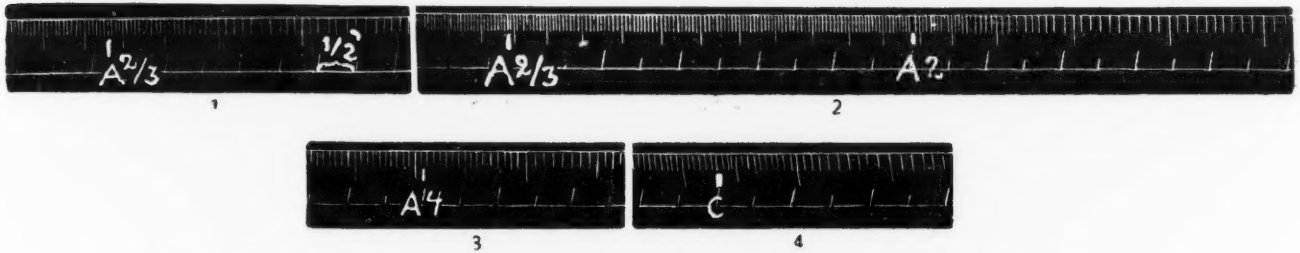


Fig. 13. Perfusion of rat hind limb. Adrenaline (A) in γ came diluted before entering into artery approximately to 2.5 ml Ringer solution, 2: Ringer with Chlor-trimeton (C) 1×10^{-6}, 3: Ringer with C 1×10^{-5} and 4: Ringer with C 1×10^{-4}

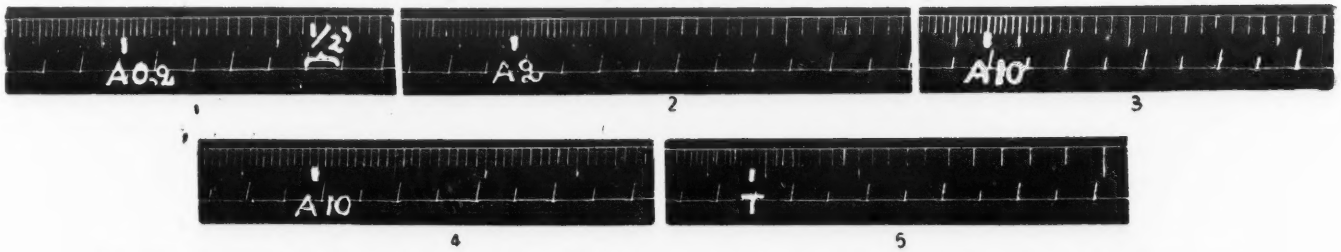


Fig. 14. Perfusion of rat hind limb as Fig. 13. 2: Ringer with 1×10^{-6} Thephorin (T), 3: Ringer with 4×10^{-5} T; 4: Ringer with 1×10^{-4} T and 5: Ringer with 0.5×10^{-3} T

The results do not support the part played by the peripheral circulation in increasing the blood pressure rising effect of adrenaline caused by chlor-trimeton. However it should be noticed that the sympatholytic effect of chlor--trimeton is noticeably weaker than that of thephorin.

Haley and Andem (1950) investigated locally the effect of several antihistaminic drugs upon the mammalian precapillary sphincters. All were strong vasoconstrictors, chlor-trimeton one of the strongest. The effects were already brought out in concentrations of a similar size to the doses given to humans. But if we consider that in our experiments 0.5×10^{-3} thephorin corresponds to 500 mg/kg and 1×10^{-4} chlor-trimeton to 100 mg/kg in rat, to produce vasoconstriction with antihistaminics the concentrations that are necessary in rat hindleg preparation are much bigger; with thephorin they are lethal, with chlor-trimeton toxic or sublethal. The blood pressure effect of adrenaline is mainly caused by constriction of the splanchnic vessels, but in this region also anthisan and thephorin have adrenolytic effect when applied locally (Haley and Harris 1949). It should be remembered that Haley and Andem (1951) ascertained that typical adrenergic blocking agents also produced vasoconstriction after topical application to rat mesoappendix.

VII. EFFECT OF ANTIHISTAMINICS ON PULMONARY EDEMA DUE TO OTHER CAUSES THAN ADRENALINE

Apart from the injection of massive doses of adrenaline, experimental pulmonary edema has been shown to be caused in many other ways. The ligation of the aorta (Welch 1872; Sahli 1889; Kotowschtschikow 1919) and the disturbance in the working capacity of the right and left ventricle, such as the compression of the left ventricle, produce it (Welch 1872). Although with excessive hydremia caused by injecting a large amount of saline solution it is difficult to produce pulmonary edema (see Wiggers 1939), it succeeds after the cutting of the vagus, an action which in itself can produce it (Kraus 1913; Brunn 1933; Farber 1937 a, b). Speedy intracarotid infusion of saline regularly causes pulmonary edema, and this has been investigated by Luisada and Sarnoff espicially (1944 and 1946 a, b). Death caused by irritating gas is a consequence of pulmonary edema and circulation collapse (Lewin 1908). These have been investigated recently by Halpern, Cruchaud, Vermeil and Roux (1950). In the year 1949 Koenig and Koenig (a, b) produced experimental pulmonary edema by injection of ammonium salts. The above-named investigators and their co-workers have also shown that experimental alkalosis causes pulmonary edema (Koenig, Schildkraut, Stahlecker and Koenig 1952). α-naphtyl-thiourea given intraperitoneally causes pulmonary edema in rats (MacKenzie and MacKenzie 1943; Halonen and Hakkila 1952). Pulmonary edema produced centrally by cardiazol and veratrine has already been mentioned earlier. It must also be remembered that besides adrenaline, some other sympathomimetic amines can bring out pulmonary edema. According to Riechert and Schmieder (1941) this is usual when lethal doses of cobefrine, icoral (base B) and synephrine are given to rats.

A. BILATERAL CERVICAL VAGOTOMY

There are many investigations into bilateral cervical vagotomy, several of which were made as early as the last century (see Schafer 1919). In the year 1937 (a) Farber summarizes the literature to date and says that death after bilateral vagotomy is caused "because of (a) aspiration pneumonia (vagus pneumonia); as a consequence of laryngeal paralysis, mouth secretions and food are aspirated causing an acute lobular pneumonia. (b) Slow asphyxia, secondary to laryngeal paralysis. With inspiration there is a falling together of the thyro-arytenoid ligaments and the arytenoid cartilages. (c) Neuroparalytic pulmonary congestion, as a consequence of the loss of the tonic vasocontrictor action of the vegasympathetic nerves". However, according to Farber (1937 a, b) laryngeal paralysis is not an essential factor in the production of severe pulmonary edema in the rabbit and guinea-pig. He considers that the most important cause is the loss of the innervation of the lungs, that is followed by serious alterations in the dynamics of the pulmonary circulation. These alterations cause blood congestion and pulmonary edema. The asphyxia that arises and the increasing capillary permeability aggravate the situation. Reichsman (1946), who in his great survey covering the last 150 years presents as explanation findings on the pathogenesis of pulmonary edema caused by bilateral vagotomy, comes to the conclusion in his experiments performed with rats that the most important factor in this sort of edema is inspiratory obstruction. The findings of Lorber (1939) also speak on behalf of this, although he acknowledges that circulatory failure might also be a factor of some importance. Weiser (1932) concluded that vagotomy caused increased permeability of the pulmonary capillaries because unilateral vagotomy in rats increased the diffusion of the intratracheally induced dye more from the lung which had been deprived of its vagal innervation than from the control lung. Because ergotamine tartrate blocks pulmonary edema after vagotomy, Riechert (1951) considers this as proof of the part played by the sympathicus in the permeability promoting action in the pulmonary circulation area.

Plester and Rummel (1951) were unable to show the effects of synopen (2 mg/kg), phenergan (4 mg/kg) or antistine (10 mg/kg) on pulmonary edema after vagotomy in rats and guinea-pigs. On

the other hand after giving dihydroergotamine to guinea-pigs the edema was slighter. In the following the effect of chlor-trimeton on the course of the pulmonary edema after vagotomy has been investigated in rats and guinea-pigs.

METHODS AND RESULTS

Rats were first used in the test. These were white males, weighing 120 — 150 gm. Bilateral cervical vagotomy was made under ether anesthesia. Respiration slowed down after cutting the vagi and became deep, all auxiliary muscles being used vigorously. At the same time a strong inspiratory crow was heard in most of the animals, which Reichsman (1946) has explained by using a laryngoscope as being due to the drawing together of the vocal chords at the end of inspiration. Within three days nearly all the animals were dead and in all the dead animals strong pulmonary edema and bloody mucus in the respiratory passages was found. Before death bloody froth came from the nostrils of the animals. At autopsy it was found that both auricles were full of blood and in the right ventricle there was also a moderate amount of blood. The results are shown in Table No. 14. The group given chlor-trimeton could

Table 14

THE EFFECT OF CHLOR-TRIMETON ON THE PULMONARY EDEMA IN RATS CAUSED BY BILATERAL CERVICAL VAGOTOMY. CHLOR-TRIMETON 10 MG/KG SUBCUTANEOUSLY 15 MINUTES BEFORE VAGOTOMY AND THEN EVERY 8 HOURS. ETHER WAS USED AS ANESTHETIC

	Number of Rats	Average Body Weight g	Average Lung Weight %	Dead Within 36 Hours. Survival Time Hours
Vagotomy	10	136	1.52	2, 2, 4, 5, 6, 7, 19, 20 and 36
Chlor-trimeton+ Vagotomy	10	141	1.60	2, 2½, 3, 4, 6, 8, 24 and 30
Controls ..... (Decapitated)	6	150	0.80	

not be seen to differ from the control group. When comparing the weights of the lungs of the vagotomised animals with the corresponding weights of normal decapitated animals a clear difference is observed.

The same test was made with male guinea-pigs, weighing 450 — 600 gm. As anesthetics for the operation 0.75 g/kg of urethane intraperitoneally and a little ether were used. The physical signs in these animals largely resembled those displayed in rats. Strong pulmonary edema was developed in all animals and this led to death within 3 — 6 hours. No evidence of pleural or pericardial effusion was found. The results are set out in Table No. 15. The animals given chlor-trimeton in no way differed from the others.

Table 15

THE EFFECT OF CHLOR-TRIMETON ON THE PULMONARY EDEMA IN GUINEA-PIGS CAUSED BY BILATERAL CERVICAL VAGOTOMY. CHLOR-TRIMETON 10 MG/KG SUBCUTANEOUSLY 15 MINUTES BEFORE VAGOTOMY. URETHANE 0.75 MG/KG INTRAPERITONEALLY AND A LITTLE ETHER USED AS ANESTHETICS

	Number of animals	Average Body Weight g	Average Lung Weight %	Survival Time Hours
Vagotomy	6	540	1.7	4.8 (3.3 — 7)
Chlor-trimeton+ Vagotomy	6	508	1.8	5.1 (3 — 7)
Controls ..... (Decapitated)	5	460	0.8	

DISCUSSION

Both in rats and guinea-pigs pulmonary edema was as a rule a consequence of bilateral cervical vagotomy. Because the symptoms of those animals given chlor-trimeton and the autopsy findings did not differ from the controls it was considered that the number of animals taken into consideration was sufficient to enable it to be said that chlor-trimeton at least in these tests has no effect on changing permeability. For the factors in pulmonary edema follow-

ing upon vagotomy were, (1) neurogenic vasodilatation in the lungs and following upon it or independent of it an increase in capillary permeability or (2), inspiratory obstruction: so at least one factor is the increased permeability or the changing of circumstances such as the transsudate shifts from the pulmonary capillaries into the alveoli. If, in fact, the cause was inspiratory obstruction, as Reichsman (1946) explains, negative intra-alveolar pressure would follow and this would tend to overcome the osmotic pressure in the pulmonary capillaries.

B. AMMONIUM CHLORIDE

Windle, Koenig and Jensen (1946) noticed first that a small over-dose of ammonium chloride given intraperitoneally to guinea-pigs caused pulmonary edema. When investigating this phenomenon more accurately Koenig and Koenig (1949 a) showed that this also happened with rats and cats. The best way was to give the substance intraperitoneally. If the dose was too large or its effect came out too quickly, as when given into the vein all at once, death followed from cardiac failure or more frequently from respiratory failure, before the appearance of lung changes. They believe that the pulmonary edema is a specific action of ammonium moiety, and is not related to the acidosis produced by ammonium chloride. They considered pulmonary vascular spasm and a change in pulmonary capillary permeability to be the most important factors (Koenig and Koenig 1949 b). According to Sarnoff and Kaufman (1951), the most important factor would be left ventricular failure. Left auricular and caval vein pressures rose significantly in all experiments. Tests with dogs show that pulmonary edema is a close consequence of impulses travelling through sympathetics to the peripheral vascular bed, increased peripheral vascular resistance as well as the shift of blood from periphery to lung (Sarnoff 1951). This would be a factor in the rise of pulmonary capillary pressure. Adrenergic blocking agents prevent ammonium chloride pulmonary edema in various animal species (Mackay *et al.* 1949). Halonen and Hakkila (1952) could not show a protective effect with morphine, eliradon, methadone, ergotamine tartrate and hexamethonium iodide, in regard to guinea-pigs. Winter (1949) did not produce any change with phenergan in the poisoning picture of rats and guinea-pigs,

but in his experiments this antihistamine drug also had no effect upon adrenaline lung edema.

In this study the effect of chlor-trimeton and thephorin on the pulmonary edema caused by ammonium chloride in rats and guinea-pigs has been investigated.

METHODS AND RESULTS

In the experiment white female rats (100 — 140 g) and female guinea-pigs (250 — 360 g) were used. The doses used are explained in Table No. 16. Koenig and Koenig (1949 a) recommended for the

Table 16

THE EFFECT OF CHLOR-TRIMETON (C) AND THEPHORIN (T) ON AMMONIUM CHLORIDE PULMONARY EDEMA. AMMONIUM CHLORIDE (6 % WATER SOLUTION) WAS INJECTED INTRAPERITONEALLY ALONE OR 15 MINUTES AFTER ANTIHISTAMINICS 10 MG/KG SUBCUTANEOUSLY

	Mortality Ratio	Average Body Weight g	Average Lung Weight % [1]	Median Survival Time Minutes [2]
Rats:				
Ammonium Chloride 400 mg/kg	8/14	118	1.9	50
C + Ammonium Chloride 400 mg/kg	7/16	124	2.0	45
T + Ammonium Chloride 400 mg/kg	10/16	113	1.9	58
Controls	0/6	134	1.0	
Guinea-pigs:				
Ammonium Chloride 500 mg/kg	3/5	273	1.1	16
C + Ammonium Chloride 500 mg/kg	2/5	300	1.0	11
Ammonium Chloride 700 mg/kg	4/5	313	1.2	10
C + Ammonium Chloride 700 mg/kg	3/4	345	1.1	10
Controls	0/4	340	0.7	

[1]) Average lung weight in experimental groups includes only those animals which succumbed after injection. Control animals were sacrified under ether anesthesia.

[2]) Excluding those surviving indefinitely.

rat 400 mg/kg and for the guinea-pig 500 — 700 mg/kg of ammonium chloride, given intraperitoneally. In our experiments these doses had a mortality effect of half or more. Rats especially had a strong pulmonary edema. Its degree corresponded roughly to that caused by subcutaneously injected adrenaline. On the other hand in the guinea-pigs the edema was smaller. The weight of the lungs was only a little over 1 % of the body weight, although after adrenaline it was about 2 %. The groups that had got antihistaminics did not differ essentially from each other. Koenig and Koenig (1949 a) have explained the picture of the ammonium chloride pulmonary edema minutely and our observations as to the cause of the poisoning and autopsy findings corresponded to theirs. The only symptom that appeared to be strongest in those given chlor-trimeton was exophthalmos in rats, but its degree has not been measured in any way.

DISCUSSION

Our experiments are in concordance with the investigations of Winter (1949). Antihistaminics that change the course of adrenaline pulmonary edema do not seem to effect pulmonary edema induced by ammonium chloride. Neither were Halonen and Hakkila (1952) able to show that the substances that they investigated had effect, although they had effect on pulmonary edema caused by adrenaline. Because the causative mechanism of ammonium salts induced lung edema has still not come in for much investigation, it is difficult to form any conclusions for the failure of antihistaminics to effect this type of pulmonary edema. Because in guinea-pigs especially the pulmonary edema was relatively slight this indicates that the most important death factor would be the toxic effect directed to the heart or elsewhere.

VIII. EFFECT OF CHLOR-TRIMETON ON INCREASED PERMEABILITY PRODUCED BY DRUGS OTHER THAN HISTAMINE

In many experiments it has been shown than it is a characteristic of antihistaminics to have the ability to block the increasing of capillary permeability caused by histamine. This also applies to the effects of histamine on other tissues. Thus antihistaminics prevent the swelling up and absorption of water of the muscle caused by histamine (Halpern and Reuse 1949). Halpern (1950) considers that the most important property of antihistaminics is their ability to decrease the increased capillary permeability. Halpern, Cruchaud, Vermeil and Roux (1950) also regard the preventing of adrenaline induced pulmonary edema as being due to the decreasing of permeability. Phenergan also prevented the production of experimental orthostatic albuminuria in rabbits (Hamburger, Halpern and Neel 1948). Because of this Halpern has put forward the view that phenergan may have some direct effect on capillary permeability apart from its effect as a histamine antagonist. Because, for one reason or another, the increasing of capillary permeability plays an essential part in the causative mechanism of adrenaline pulmonary edema, it was felt that it would be interesting to ascertain if chlor-trimeton, which contrary to phenergan increases adrenaline toxicity, could be shown to have the property of increasing permeability.

Usually the increased capillary permeability has been investigated in animals by injecting a dye of large molecular structure, such as trypan blue, into the circulation, and comparing the penetration of the dye into the skin, which usually follows intradermal injection of histamine (Menkin 1936). Although antihistaminics prevent the effect of histamine, in this experiment benadryl

and anthisan for example when given subcutaneously do not prevent the effect of other such substances that in this experiment cause the extravasation of the dye (Last and Loew 1947). Among such agents there were trypsine, snake venom, staphylococcus toxin, pantocaine and codeine. Nor did thenylene or pyrrolazote have any effect on the intensity of edema and trypan blue concentration produced by the application of xylene to the skin (Rigdon 1949). Although trypsine, for example, has been said to liberate histamine (Kellaway 1939), the fact that antihistaminics do not prevent the rise in permeability caused by these substances goes to prove that their property of increasing capillary permeability is not based upon the liberation of histamine. On the other hand, if antihistaminics directly decreased permeability they should decrease the increased permeability caused by a variety of agents which do not contain or liberate histamine.

METHODS AND RESULTS

The local staining of a test side in the skin of the rabbit following the intravenous injection of 1 % trypan blue was employed as a means of demonstrating changes in capillary permeability. Trypsine (E. Merck, Darmstadt) and pantocaine hydrochloride were chosen as agents for increasing permeability. The fur of a number of adult rabbits was removed with "Veet" hair removing cream (Dae Health Laboratories Ltd., London) from both sides of the flanks and stomach area one day before. Trypan blue was diluted with water, other drugs with saline. Directly before giving trypan blue, trypsine was injected intracutaneously into five rabbits at two points on one side. At corresponding points on the other side trypsine and chlor-trimeton were injected in the same syringe. The total amount was always 0.2 ml, the final concentration of chlor-trimeton was 2×10^{-5} and of trypsine 0.2 mg/ml in both cases. The same experiment was made with five rabbits, but instead of trypsine, pantocaine was used, the concentration of which was 0.3 mg/ml. The results showed clearly within the first half hour. The reaction was compared for the last time 24 hours after the injection. In noting the results the following symbols were used: 0 = negative response, + ? = doubtful, + = pale homogeneous blue spot and + + = deep blue homogeneous spot.

In those given trypsine the reaction was + + for two, + for two and 0 for one. With pantocaine the result was + + for three, + for one and + ? for one. In both sides there was a clearer increase of permeability at the more ventral injection point. In two rabbits from each group the response appeared weaker at points into which chlor-trimeton had also been injected, but the difference was so small that the reaction could not be differentiated upon the scale. Chlor-trimeton injected alone had no effect in the above-mentioned concentration.

Trypan blue was injected intracutaneously into four rabbits as 0.5 % solution. This was injected together with 2×10^{-5} chlor-trimeton on the same side at another point; and similarly on the other side, but in addition to the first there was also adrenaline (1×10^{-4}). The concentrations reported were calculated to the total fluid capacity which was always 0.2 ml and was injected in the same syringe.

After two hours the blue spot was about 1.5 cm in diameter, after four hours 2 cm, and after twenty hours 3 cm. The addition of chlor-trimeton did not change the size of the spot nor the intensity of the colour. The presence of adrenaline prevented the spreading of the dye, so that after four hours for example the diameter of the spot was hardly 1 cm. And in this case also the addition of chlor-trimeton did not alter the result.

DISCUSSION

The results in the first part of the test strengthen the view that trypsine and pantocaine here do not have effect through histamine. Also chlor-trimeton does not seem to have effect on capillary permeabality increased in this way. Antihistamine substance was injected here intracutaneously and it was found in the preliminary tests that when given in this way it prevented the effect of histamine injected into the same point. Hensen (1950) found in a study of the effects of locally applied antihistaminics against the response to intracutaneous histamine, the dye infiltration to be diminished by pyribenzamine, decapryn and anthisan, but thephorin was found to be completely ineffective. A dose given intravenously did not change the result.

The result in the second part of the test corresponds to the first part. In this the effect can be considered as being on the intercellular cement of tissue cells, whereas instead in the former it is more a question of the rise of capillary permeability. Rocha e Silva and Dragstedt (1941) ascertained that hyaluronidase does not produce a trypan blue reaction because it just effects the intercellular cement. It has been determined that the spreading effect of hyaluronidase is increased by adrenaline when injected into the human skin (Kirby *et al.* 1949). Nothing pointing to this hyaluronidase property was discovered from chlor-trimeton in the skin of a rabbit. There is on the contrary evidence that antihistaminics suppress the effect of hyaluronidase *in vivo* (Mayer and Kull 1947; Elster *et al.* 1949). The experiments do not speak for the direct effects of chlor-trimeton on permeability.

IX. EFFECT OF ANTIHISTAMINICS ON MYDRIASIS PRODUCED BY ADRENALINE

In the year 1898 Lewandowsky (1898 and 1899) showed that suprarenal extract had the same effect on the mammalian eye as sympathetic stimulation. Then there happens, amongst other things a contraction of the pupil dilator muscle, the consequence of which is mydriasis. This is also used for the biological assay of adrenaline especially after the removal of the superior cervical ganglion, when the sensitivity of the "denervated" iris is greatly increased (Meltzer and Auer 1904). One much used method is to measure the diameter of mouse's pupil after the injection of the substance under investigation. Pulewka's (1932 and 1939) standardisation method of atropine was based just upon this. Recently Forst and Deininger (1952) have made adrenaline determinations with mice from which the superior cervical ganglion had been excised.

Frölich and Loewi already discovered in 1910 that cocaine causes increased dilatator response to adrenaline. The same applies to thyroxine, and thyroxine sensitization is probably the reason why the pupil of a patient with Basedow's disease expands considerably with the administration of 1 : 1000 adrenaline solution drops into the eye (Goetsch 1922). Ergotomine does not prevent adrenaline mydriasis in the frog eye (Dominguez and Solomjan 1926). Dibenamine prevents mydriasis induced by cervical sympathetic stimulation in the cat (Nickerson and Goodman 1947). The miotic effect of dibenamine may rather follow from adrenergic blockade than from the direct stimulation of the smooth muscle, as is the case with ergot alkaloids (Nickerson 1949). Priscoline has very slight ability to prevent adrenaline mydriasis (Chess and Yonkman 1946) and neither has yohimbine a strong one (Stilwell and Jeremias 1944).

In so far as antihistaminics have been investigated, they have a dilating effect on the pupil of the eye or they have no effect on the muscles of the iris (see Haas 1951). Their effect is considered to be mostly due to the presence of atropine properties. Adrenaline put into the eye locally accelerates the action of previously applied benadryl upon the pupil but does not increase its effect. Orally administered benadryl in doses of up to 400 mg daily does not influence the size of the pupil of the eye (Harris *et al.* 1946). Locally applied antihistamines irritate the eye and cause slight conjunctivitis. One of the slightest irritants is considered to be antistine, although even it has some disadvantages, but the size of the pupil however does not change after the application of 0.5 % solution (Schlaegel 1949). Thephorin as 3 % solution had no effect when instilled into the conjunctival sac of cats, whereas atropine was effective in a concentration of 0.001 % (Lehman *et al.* 1949).

Because we were especially interested in the change in pupil reaction caused by adrenaline, we investigated the effect of chlortrimeton and thephorin in such doses, which in themselves had no effect upon the eye of the test-animal.

METHODS

In the experiments male white mice, weighing 25 — 30 gm and accustomed to experimentation, were used. They were not kept without food. The measurement of the pupil was made according to Pulewka's method (1932 and 1939) in a half-darkened room. In the preparation microscope used there was a magnification of 16 and the size of the pupil was compared with the ocular scale. The medicines were injected subcutaneously with a very thin needle. The tests were made for the most part as cross-over tests. For the sake of clarity only the maximum pupil dilatation is shown. Before injection the size of the pupil was measured at intervals of 10 — 15 minutes until there were no changes to be observed. Then the antihistaminic (10 mg/kg) was injected and fifteen minutes later 1 — 4 mg/kg of adrenaline, or else adrenaline was injected without preliminary antihistamine treatment. Ten minutes after the adrenaline injection the first measurement was made, then once every fifteen minutes for 1½ — 2 hours and

later less frequently. The final measurement was sometimes made as much as five hours later. All the tests were carried out between 9 and 15 o'clock so as to eliminate the possible variations of the day.

RESULTS

Chlor-trimeton and thephorin in doses of 10 mg/kg did not have any effect on the size of the pupil. From Table No. 17 it can be seen that the iris of the mice used was relatively

Table 17

THE EFFECT OF CHLOR-TRIMETON AND THEPHORIN GIVEN TOGETHER WITH ADRENALINE ON THE SIZE OF THE PUPIL OF MOUSE EYE. THE ANTIHISTAMINICS WERE INJECTED 15 MINUTES BEFORE ADRENALINE, BOTH DRUGS SUBCUTANEOUSLY

Number of Animals n	Adrenaline mg/kg	Antihistam.		Size of Pupilla Mean		Dilatation of the Pupil Mean	$\sigma (\bar{X}_1 - \bar{X}_2)$*
		Drug	mg/kg	before $\bar{X}_1$	after $\bar{X}_2$		
9	1.0	—	—	0.42	0.53	0.11	0.03
9	1.5	—	—	0.33	0.44	0.11	0.04
9	2.0	—	—	0.32	0.38	0.06	0.02
10	3.0	—	—	0.39	0.53	0.14	0.05
19	4.0	—	—	0.39	0.62	0.23	0.05
9	3.0	Chlor-trimeton	1.0	0.31	0.55	0.24	0.04 [1]
15	3.0	„	5.0	0.37	0.92	0.55	0.06 [2]
13	2.0	„	10.0	0.35	0.90	0.55	0.07 [2]
9	2.0	The-phorin	10.0	0.38	0.80	0.42	0.04 [2]
10	3.0	„	10.0	0.28	0.81	0.53	0.07 [2]
15	4.0	„	10.0	0.35	0.98	0.63	0.06 [2]

[1] The effect of antihistaminic is not significant, because the difference between the means 0.24 deviates too little from the observation error 0.1.

[2] The effect of antihistaminic is highly significant, $P<0.001$.

* The standard deviation of the difference between two means was calculated from the formula $\sigma (\bar{x}_1 - \bar{x}_2) = \sqrt{\sigma (\bar{x}_1)^2 + \sigma (\bar{x}_2)^2}$, in which $\sigma (\bar{x}_1)$ and $\sigma (\bar{x}_2)$ are the standard deviations of the means. The standard deviation of the mean is calculated from the formula $\sigma (\bar{x}) = \frac{\sigma(x)}{\sqrt{n}}$. The standard deviation of the single observation $\sigma (x)$ is estimated by the formula $\sqrt{\frac{\Sigma (x_y - \bar{x})^2}{n-1}}$ where $\Sigma (x_y - \bar{x})^2$ is the sum of the squares of the deviation of the several x values from $\bar{x}$.

insensitive. 3 — 4 mg/kg of adrenaline caused only moderate mydriasis. The maximum mydriasis reading was 1.5 — 2.0 scale units. Administering antihistaminics increased the reaction strongly and the increasing effect of antihistaminics on adrenaline mydriasis can be considered as highly significant when the dose is 10 mg/kg. When investigating the effect of smaller doses of chlor-trimeton it was found that the case is the same when the dose is 5 mg/kg, but with a dose of 1 mg/kg the result is no longer sure in so far as a tenth of the scale is regarded as the limit of error observation. At the same time antihistamines, while increasing mydriasis, also lengthened its duration to 3 — 5 hours, whereas the original condition was normally obtained in 1 — 1½ hours. In mice which had been given chlor-trimeton, the adrenaline effect appeared also as strong general symptoms and there was one case of death in the group given 3 mg/kg of adrenaline after 5 mg/kg of chlor-trimeton.

DISCUSSION

Mydriasis is the only adrenaline response that is increased by both the antihistaminics investigated. The result cannot be a pure summation, for these antihistaminics even in doses of 20 mg/kg given subcutaneously, did not effect mydriasis in some of the experiments or the effect was quite slight with chlor-trimeton. It is known "that the mydriatic effect of atropine is generally assisted by reciprocal (central) stimulation of the sympathetic innervation, so that the atropinized pupil contracts somewhat if the sympathetic innervation is severed" (Sollmann 1948). Because antihistaminics also possess atropine effect (see p. 16) one might therefore think that adrenaline here only sensitizes the anti-acetylcholine effect of the investigated antihistamines in the pupil of the mouse. It seems impossible that in regard to thephorin for example there could be a specific change in the adrenaline effect because, as far as is known, true sympatholytic agents have not been shown to sensitize adrenaline mydriasis (see Nickerson 1949). On the other hand antihistaminics have effect upon the nictitating membrane, which has only anatomical sympathetic innervation, in the same way as upon the blood pressure (see p. 50).

X. EFFECT OF ANTIHISTAMINICS ON HYPERGLYCEMIA PRODUCED BY ADRENALINE

One of the most typical effects of adrenaline appears in the carbohydrate metabolism. As early as 1901 Blum showed that adrenaline injected caused glycosuria in warm-blooded animals. This again is a consequence of hyperglycemia (Zuegler 1901; Metzger 1902). Miculicich in 1912 determined that ergotoxine reduced the rise in blood sugar induced by adrenaline in the rabbit. The same blocking activity has been shown to be typical of all ergot alkaloids possessing adrenaline blocking activity (Rothlin 1946 and 1947). It has been shown that many other sympatholytic substances have the same effect, although there are great differences in the reactions of different types of animals [priscoline (Harvey, Wang and Nickerson 1952), regitine (Hecht, Crandall and Samuels 1950), yohimbine (Nitzescu 1928)].

In the year 1948, Chen and Clarke investigated the effect of benadryl given subcutaneously on the blood sugar increasing ability of adrenaline given in the same way. Benadryl increased the response although not significantly. Later the effects of antihistaminics in this connection have been shown in other investigations. In the experiments of Susina and Unna (1951) intravenously given benadryl and chlor-trimeton increased hyperglycemia in a dog, but thephorin decreased it. Komrad and Loew (1951 b) investigated a great number of antihistaminics on the adrenaline hyperglycemia of the rabbit and partly of the dog. The ability of the substances investigated to block adrenaline hyperglycemia in rabbits is related to the potency of these agents as measured by their antagonism to the effects of histamine on the bronchioles and on the ileum in guinea-pigs. In dogs some strong antihistamines did not cause the blocking of the adrenaline effect, although it did come out in rabbits. Chlor-trimeton for example was one of

these that potentiated the effect of adrenaline. It did not effect the fasting blood sugar level. The rate of the muscle glycogenolysis was not affected by the drugs because adrenaline induced lacticacidemia was not decreased. The site of the action was therefore only the liver. Among others, cocaine, atropine, and nembutal had no effect. Benadryl given orally to rats in a large dose (30 mg/kg) increases blood sugar, but pyribenzamine hardly at all (Tepperman *et al.* 1951).

The experiments referred to concerning the effects of antihistaminics on adrenaline hyperglycemia have mainly been performed with rabbits and/or dogs. Because type differences have been observed and because, at least in the rat, chlor-trimeton and thephorin change adrenaline toxicity, investigations were made to see if they differed from each other in this respect also, and in what manner. Komrad and Loew (1951 a) consider that the effectiveness of adrenergic blocking drugs in diminishing or blocking adrenaline induced hperglycemia in rabbits is related to their potency as measured by the antagonism of other adrenaline effects. On the other hand on the basis of earlier results and the work of Harvey, Wang and Nickerson (1952), there are no such correlations, at least when comparing blood sugar and pressor responses. "It seems likely, therefore, that the" hyperglycemia receptors "are different from the" pressor receptors "or that a completely different mechanism of action is involved in the suppression of these two responses".

METHODS

In the experiment female rats, weighing 200 — 300 gm, acclimatized to the withdrawal of blood, were used. They got different solid foods except for the 24 hours before the experiment, when they were given only casein and water. With this method the most even blood sugar curves were obtained with alloxan diabetic rats (Vartiainen 1952). During the experiment itself nothing was given. The experiment was performed on the same rats as a cross-over test with an interval of at least four days. The blood was taken from the tip of the warmed tail and sugar determinations made according to Creselius-Seifert principle using an Eel-electrophotometer and green filter No. 404. Because it was diffi-

cult to determine with this method the blood glucose level below 100 mg %, experiments in which pure antihistaminic was given were made according to Hagedorn-Jensen. 10 mg/kg (1 : 100 water solution) of antihistaminics were injected subcutaneously and 15 minutes later adrenaline subcutaneously as far as possible from the first — 0.1, 0.4, and 0.8 mg/kg in saline diluted to 1 : 20,000, 1 : 5,000 and 1 : 2,500. Blood samples were taken before and ½, 1, 2, 3½, and 6 hours after the adrenaline injection. Blood sugar responses were plotted and the areas obtained under the curve were compared statistically. Then the sixth hour values were discounted.

RESULTS

The antihistamines used did not have effect in doses of 10 mg/kg on the blood sugar level. After 0.4 mg/kg of adrenaline the area of hyperglycemia was smaller when pre-treated with chlor-trimeton (Table No. 18). This is the only significant result

Table 18

THE EFFECT OF CHLOR-TRIMETON (C) AND OF THEPHORIN (T) ON ADRENALINE HYPERGLYCEMIA IN RATS

Number of Tests n	Adrenaline subcutan. mg/kg	Antihistaminics subcutan. 10 mg/kg	Max. Rise Mean		Mean Area of Hyperglycemia	
			mg. %	% of init. Value	Mg % × Hour	$\sigma(\bar{x})$ [1]
15	0.4	—	94	80	632	22
8	„	C	84	71	554	20 [2]
8	„	T	84	68	595	26
4	0.8	—	80	60	602	37
4	„	C	103	93	626	26
4	„	T	82	75	570	20
4	0.1	—	22	19	423	22
4	„	C	37	36	424	22
4	„	T	29	26	431	20

[1] $\sigma(\bar{x})$ is the standard deviation of the mean and it is calculated as in the previous chapter.

[2] The only significant difference from the control group, $P<0.05$.

($p < 0.05$). Thephorin did not change the reaction significantly. It has been shown that 0.1 mg/kg of adrenaline given subcutaneously to rats increases glycogen in the liver but decreases it in the muscle, when again with larger doses this is not brought out or the glycogen amount of the liver decreases (Cori and Cori 1928 and 1929; Eadie 1930). Because the mechanism of hyperglycemia after a large and a small adrenaline dose would thus to some extent be different, preliminary tests were made with smaller and larger doses. There were no differences in those which had got 0.1 mg/kg of adrenaline after antihistaminics. Distribution in the control group of those which had got 0.8 mg/kg of adrenaline was very great, and nothing can be said on the basis of this result. However it appears that chlor-trimeton here would not prevent the effect of adrenaline. If the maximum values of hyperglycemia are compared, chlor-trimeton and thephorin do not differ from one another in the first group, in which they seem to block adrenaline response slightly. However in both other groups the effect would be contrary.

DISCUSSION

In this work neither chlor-trimeton nor thephorin seems to have at least any large effect on adrenaline hyperglycemia. Indeed it was found that chlor-trimeton weakened the effect of 0.4 mg/kg of adrenaline significantly when the mean area of hyperglycemia was the criterion. If as criterion the maximum rise in blood sugar is used, as Komrad and Loew (1951 b) suggest, the results with thephorin and chlor-trimeton do not differ from each other and only a little from the control value. Although chlor-trimeton in the two last groups of the table seemed to increase the reaction, the result is very uncertain because in these groups there were only four experiments. The result differs from that which Susina and Unna (1951) got with dogs. It is true that they gave adrenaline into the vein and here it was given subcutaneously. The effect of a dose given directly into the circulation is quicker and it may be thought that then a near toxic effect might appear. The weakening effect of chlor-trimeton also did not appear in our experiments when a larger dose of adrenaline was given. Because the adrenolytic effect could be shown with chlor-trimeton, the result can be com-

pared with Komrad and Loew's (1951 b) results from rabbits which were given 4 γ/kg of adrenaline intravenously. In the work of Chen and Clarke (1948) also, benadryl given subcutaneously blocked the effect of adrenaline, although in our experiments benadryl increased the toxicity of adrenaline, as did chlor-trimeton. Ephedrine also blocks the hyperglycemic action of adrenaline (Ellis 1951; Komrad and Loew 1951 b), although these two agents synergize with respect to vasomotor actions. In this connection it may be mentioned that benadryl potentializes the adrenaline induced oxygen consumption of rats (Fabinyi — Szebehely and Szebehely 1952 b).

XI. ANTIHISTAMINICS AND ADRENALINE IN ANAPHYLAXIS

A.-M. Staub (1939 a) was able to demonstrate that 929 F had some anti-anaphylactic effect. Later in many experiments this feature has been show to be common to all antihistaminics (see Reuse 1949 b). It may be observed that the necessary antihistaminic dose for blocking anaphylactic shock is larger than that necessary for blocking histamine shock. The guinea-pig is the test-animal most used in anaphylactic shock. It is most easily sensitized, its shock symptoms are typical and usually end in death. Death itself in acute shock, in which the antigen has been injected into the circulation, is due to suffocation (Doerr 1950). Then the smooth muscles of the fine bronchioles contract and the swelling up of the mucous membrane and the secretion of mucus worsen the situation. On the other hand it is known that as early as 1905 Kaplan recommended adrenaline for the treatment of asthma attacks. Adrenaline does not effect the normal bronchioles greatly, but in the experimental bronchial spasm it causes quick dilatation (Janushke and Pollak 1911). In this respect adrenaline is the strongest substance known (Gold 1945). In preventing histamine bronchial spasm, apart from antihistamines only adrenaline has effect in the same concentrations (Haas 1951). This also applies to experiments made with the isolated lung (Land *et al.* 1949). The results with antihistaminics in bronchial asthma are very variable and at least in part are probably due to the fact there are other than allergic agents in the pathogenesis of the illness. With thephorin among others, the favourable results obtained vary between 20 — 80 % (Criep and Aaron 1948; Rosenberg and Blumenthal 1948).

Because at any rate antihistamine treatment is not sufficient for the most part for asthmatics, adrenaline is still used in the most difficult cases. If adrenaline liberates histamine in the organism,

antihistaminics by blocking the effect of histamine — in this case one of the two physiological antagonists — can increase the effect of adrenaline. At intervals in asthma attacks Kallós and Kallós-Deffner (1951) were able to reduce the ephedrine dose to half when thephorin was given at the same time, a dose which when given alone had no effect. Also it has been pointed out that pyribenzamine and ephedrine have a synergistic effect on bronchial asthma (Koepf *et al.* 1946).

Because chlor-trimeton and thephorin change the toxicity of adrenaline in different ways, it was thought it would be interesting to see what effect adrenaline would have in anaphylactic shock when given to guinea-pigs, which at the same time had been given a dose of the antihistaminics in question that give a definite protective effect.

METHODS

The guinea-pigs used were 500 — 650 gm males. In the experiment there were also about twenty animals brought from another laboratory and the degree of sensitivity of these was weaker than of those presented in the following. However the results obtained with them did not appear different. The guinea-pigs were sensitized with normal horse serum which was injected 0.1 ml diluted to 1 ml with normal saline. Re-injection was made intracardially after twenty days. The antihistamines and the adrenaline were injected subcutaneously with different syringes about 15 minutes before the re-injection at points distant from each other in the skin of the back.

RESULTS

The test-results are shown in Table No. 19. The degree of sensitivity was rather large, for 0.10 ml of serum already caused strong shock and with 0.20 — 0.30 ml without preliminary treatment it proved fatal. 5 mg/kg of thephorin subcutaneously in the experiments of Kallós and Kallós-Deffner (1951) protected 80 % of the guinea-pigs for 15 minutes in antigenaerosol atmosphere, from which otherwise animals die within six minutes. In the work of Feinberg *et al.* (1950), 3 mg/kg of thephorin intraperitoneally

Table 19

THE EFFECT OF ADRENALINE ON ANAPHYLACTIC SHOCK IN GUINEA-PIGS WHEN GIVEN TOGETHER WITH CHLOR-TRIMETON AND THEPHORIN. THE GUINEA-PIGS WERE SENSITIZED WITH 0.1 ML NORMAL HORSE SERUM DILUTED TO 1.0 ML WITH SALINE. RE-INJECTION WITH THE REPORTED AMOUNT OF THE SAME SERUM DILUTED TO 1.0 ML, THE ADRENALINE AND THE ANTIHISTAMINICS WERE INJECTED SUBCUTANEOUSLY 15 MINUTES BEFORE RE-INJECTION

Number of Animals	Adrenaline (1 : 10 000) mg/kg	Chlor-trimeton (1 : 8000) mg/kg	Thephorin (1 : 1000) mg/kg	Antigen ml	Anaphylactic Reaction
1				0.10	++
1				0.15	++
1				0.20	+++
4				0.30	+++, +++, +++, +++
2		0.25		0.50	0, + ?
4		0.25		1.00	+, ++, ++, +++
2			2.0	0.50	0, +
4			2.0	1.00	++, +++, +++, +++
6	0.2	0.25		1.00	0, 0, 0, 0, +, ++
6	0.2		2.0	1.00	0, 0, 0, +, ++, ++

0 = No visible shock symptoms
\+ = Masseter convulsions or quite mild bronchopasms
++ = Severe bronchospasms and protracted shock
+++ = Shock leading to deaht

protected 62 % and 3 mg/kg of chlor-trimeton 80 %. The median protective dose of orally administered chlor-trimeton was 0.370 mg/kg in the studies of Spoerlein, Makowsky, Margolin and Tislow (1951). In this experiment the antihistaminic doses used, 0.25 mg/kg chlor-trimeton and 2.0 mg/kg thephorin, had about as strong effect. After them 1.0 ml serum caused strong shock and 0.75 ml had effect although to a lesser extent. Concerning the differences obtained in anaphylactic reaction after adrenaline-antihistaminic injection, nothing much can be said, but both groups seemed to be similar. Those given chlor-trimeton and adrenaline have two + s

fewer, but there was such a difference already without adrenaline when the groups were compared to each other. It was difficult to obtain at a single occasion bigger homogeneous animal material.

DISCUSSION

Although the material is small, it seems that when guinea-pigs have been given thephorin or chlor-trimeton, adrenaline furthers to the same extent their chances of getting over anaphylactic shock. The groups can only be compared to each other, but if the effect of these antihistaminics that changes the adrenaline effect in differrent ways appeared in the blocking of anaphylactic shock, then it might be expected that the protective effect in the group given thephorin and adrenaline would be relatively weaker than in the group given chlor-trimeton and adrenaline. In such an investigation the microshock method would obviously be the most advantageous to use, as Herxheimer (1952) has recently shown. In this the same animals can be used many times. According to Halpern (1942), 0.25 — 0.50 mg/kg of adrenaline had a clear protective effect on histamine asthma in guinea-pigs. The maximum effect was 15 minutes after the subcutaneous injection. The favourable influence of adrenaline in anaphylactic shock which appears as a blocking of the spasms of the smooth muscles and of peripheral vasodilatation, can change disadvantageously when the dose is increased. Then the blood pressure and the heart rate rise, and the smooth muscles convulse and peripheral blood vessel spasm follows (Doerr 1950). If chlor-trimeton has sympathomimetic properties and thephorin the reverse, it might be supposed that after chlor-trimeton a smaller dose of adrenaline would be sufficient to produce the same as a larger dose after thephorin. Because, however, the adrenaline effect does not improve from the fixed optium dose, but worsens when the dose is increased, evalution of the results presented concerning the adrenomimetic or -lytic properties of the antihistaminics used is impossible.

XII. GENERAL DISCUSSION

When we look at the results we see that antihistamines can have either adrenolytic or adrenomimetic ability, depending upon the test objects and the circumstances. Also both properties can be lacking in them. This dependence of the difference of response upon the dose and the test object is typical of the substances that have an effect like that of the autonomic nervous system (Bovet and Bovet-Nitti 1948). It is a fact that some antihistaminics in definite doses increase the toxicity of adrenaline and noradrenaline in whole animals of various species and others decrease this. Why this occurs it is difficult to say, for amongst other things the pathogenesis of adrenaline pulmonary edema is still unexplained in spite of numerous experiments. It is difficult to see any typical difference in the different groups in the chemical structure of the antihistamines investigated.

The blood pressure response of adrenaline and noradrenaline is the only reaction besides the contraction of the nictitating membrane, in which the chlor-trimeton and thephorin investigated have effect in the same direction in poisoning tests and in regard to which, according to the literature, the effect of other antihistaminics is also in the main such as would be expected on the basis of lethality experiments of adrenaline. No specific effect on the pulmonary circulation could be shown. But the results from these tests speak on behalf of the view that the rise in blood pressure after adrenaline injection is exclusively caused by back pressure (Hamilton *et al.* 1938). Evisceration and decerebration did not change the blood pressure response. Neither did urethane narcosis nor scopolamine decrease the ability of chlor-trimeton to increase adrenaline toxicity, suggesting that the site of action was not cerebral and was not able to be changed by narcosis. Neither did adrenalectomy change the effect of chlor-

trimeton and thephorin on the LD of adrenaline, and adrenalectomy itself decreased the LD of adrenaline by only 20 — 30 % in mice.

No effect on the reactions of the heart could be proved, but in perfused rabbit heart antihistamines in a concentration of 1×10^{-6} already had a paralysing effect. When the heart was made hypodynamic with thephorin the adrenaline response was not so clear as before. This is obviously due to the perfusion fluid and other exceptional circumstances.

It is singular that the effect on the peripheral circulation in the perfused rat's hind limb is strongly adrenolytic both with chlor-trimeton and thephorin. The effect of chlor-trimeton is however weaker. In Fleckenstein's (1952) experiments, the adrenolytic effect was obtained on the rabbit ear with smaller concentrations with those substances that in our experiments decreased adrenaline toxicity and vice versa. Because after evisceration, chlor-trimeton increased the adrenaline blood pressure response in the whole animal, the results obtained in perfusion experiments cannot correspond to the real blood vessel reactions. Neither does cocaine in large doses increase the peripheral blood vessel effect of adrenaline but even decreases it (Läwen 1904), although it does increase the adrenaline blood pressure response.

The effect of antihistamine substances is specific in adrenaline pulmonary edema, for they have no effect on pulmonary edema after bilateral cervical vagotomy or ammonium chloride. Halpern, Cruchaud, Vermeil and Roux (1950) with large or very large doses of phenergan produced a protective effect in some degree to chloropicrin and phosgene, and this they considered to be due to the lowering of capillary permeability. According to Rothlin (1947 b), pitocin and natural ergot alkaloids, which are vasoconstrictors but not hydrated vasodilating ergot alkaloids, block phosgene pulmonary edema. Antihistamines in large doses are *in situ* vasoconstrictors and possibly in the aforementioned experiments of Halpern *et al.* the result obtained may be explained by the vasoconstriction caused by phenergan. The result obtained from the permeability experiments made on the skin of rabbits only goes to show that the permeability decreasing effect of chlor-trimeton, and evidently of other antihistaminics, appears especially only as a blocking of the increase of permeability caused by

histamine. However, it is not known how the causative mechanism of permeability changes in adrenaline pulmonary edema acts, and to where the effect of the substances investigated is directed.

Because chlor-trimeton and thephorin both increased adrenaline mydriasis it indicates that in this case the atropine properties appear with thephorin at least. On the other hand the reaction of the nictitating membrane followed the typical blood pressure reaction. Because chlor-trimeton and thephorin have no clear effect in adrenaline hyperglycemia it is evident that the effect of these antihistamines is directed more to the circulation system than to the metabolic rate.

It is known that adrenaline and antihistaminics are the most potent drugs against anaphylactic shock. When adrenaline was given with chlor-trimeton or thephorin the anti-anaphylactic potency of both these antihistamine substances was increased to the same degree. One would think that there would be some question of a simple summation of the anti-anaphylactic properties of the above-mentioned antihistaminics and adrenaline.

Thephorin resembled sympatholytic drugs both in the fact that its adrenolytic effect came out in blood pressure, in toxicity and in the nictitating membrane and in that it did not have any effect on the adrenaline responses of the isolated heart or on blood sugar, which, as is known, are difficult to block. Its effect on adrenaline mydriasis was sympathomimetic and in a blood pressure test no "vasomotor reversal" was produced by it.

Although chlor-trimeton resembles cocaine, the most important difference is that the last-mentioned strengthens adrenaline when injected into the portal circulation more than when injected into the femoral vein. Chlor-trimeton could not be shown to have this property. Thephorin weakened the effect of adrenaline on the blood pressure to the same degree independent of the injection point. So the effect of antihistamines cannot be directed to the inactivation of adrenaline in the liver. Cocaine may have this effect. West (1951 a) obtained the same results with methylene blue as with cocaine. This latter also has a blocking effect on amine oxidase. The effect of chlor-trimeton differs from that of thyroxine in that among other things thyroxine does not increase the toxicity of noradrenaline but chlor-trimeton does (Kroneberg and Hüter 1951).

The great divergence in the pharmacological properties of antihistaminics is surely to a large measure due to the fact that depending on the dose, now one, now another property comes to the forefront, each one however having its own special features. Of the substances investigated chlor-trimeton especially has adrenomimetic properties and thephorin adrenolytic. This appears particularly when the toxic effects of adrenaline and noradrenaline are in question and even with small doses in the blood pressure response. In experiments in which their effect on the toxicity of other sympathomimetic amines was investigated (Paasonen 1953), no effect was determined upon such substances as ephedrine, amphetamine, synephrine, and veritol (paredrinol) among others. The effect of antihistamines upon the toxicity of adrenaline and noradrenaline must be regarded as a quite specific property which at least has nothing to do with the specific histamine antagonistic action of these drugs.

From the clinical point of view, on the basis of the above, it must be noticed that when giving antihistaminics and adrenaline or noradrenaline at the same time to a patient, the effect of the latter drugs can essentially change, depending upon the properties of the antihistamine substance. The experiments presented hardly support the use of antihistaminics in the treatment of pulmonary edema and it should be noticed particularly that there is some evidence for quite unfavourable action in those conditions with some antihistaminics.

XIII. SUMMARY

The antihistaminics, chlor-trimeton, benadryl and anthisan when given subcutaneously to mice in a dose of 10 mg/kg, increase the toxicity of adrenaline, as does pyribenzamine in a dose of 1 — 4 mg/kg. Thephorin, phenergan and antistine in a dose of 10 mg/kg decrease the toxicity of adrenaline. The effect of chlor-trimeton and thephorin appears when given to mice, rats and guinea-pigs. The LD_{50} of adrenaline is about 50 % smaller than normal after chlor-trimeton and about 100 % greater after thephorin. The way in which the adrenaline is given has no significance and pulmonary edema seems to be the usual cause of death. Beginning from a dose of 1 mg/kg, chlor-trimeton increases the toxicity of adrenaline and after 10 mg/kg its effect lasts at least six hours. Cocaine, given to mice subcutaneously, increases adrenaline toxicity. Adrenaline does not change the LD of chlor-trimeton. Urethane and scopolamine have no effect on the ability of chlor-trimeton to increase the toxicity of adrenaline.

Chlor-trimeton and thephorin have a similar effect on the toxicity of noradrenaline as of adrenaline, but perhaps a little weaker.

Adrenalectomy does not alter the effect of chlor-trimeton and thephorin on adrenaline toxicity in mice.

In blood pressure experiments chlor-trimeton (1 mg/kg or more) increased and thephorin decreased adrenaline and noradrenaline responses. Decerebration and evisceration did not alter the result. The blood pressure rise in the right ventricle, in the left auricle and in the common carotid artery occurred in the same proportion when registered in cats. Cocaine increased the effect of the adrenaline blood pressure response more when injected into the portal circulation than when into the femoral or jugular vein. After chlor-trimeton and thephorin there was no difference in the in-

creasing or decreasing of the adrenaline response when using the aforementioned methods of administration. In guinea-pigs under urethane narcosis there was no difference in the blood pressure and respiratory responses between those given chlor-trimeton or thephorin and the control animals when adrenaline was given as a slow intravenous injection. Chlor-trimeton increased and thephorin decreased the adrenaline reaction of the nictitating membrane.

In the isolated frog and rabbit heart neither chlor-trimeton nor thephorin could be shown to have effect upon adrenaline reaction, but antihistamines already had a paralysing effect upon the rabbit heart in concentrations that could be regarded as ineffective in the whole animal.

Chlor-trimeton and thephorin are vasoconstrictors in rat hind limb preparation in large concentrations. In concentrations regarded as therapeutic they are adrenolytic. In this respect thephorin is the stronger.

Chlor-trimeton did not change the fatal effect of bilateral cervical vagotomy in rats and guinea-pigs when judged from the average lung weight and survival time. Chlor-trimeton and thephorin also had no effect upon the pulmonary edema and mortality after intraperitoneally administered ammonium chloride in rats and guinea-pigs.

Chlor-trimeton was without effect on increased permeability produced by trypsine and pantocaine in rabbit skin and judged after extravasation of intravenously administered trypan blue. Also chlor-trimeton had no effect on the spreading speed of intradermally injected trypan blue alone or with adrenaline.

Chlor-trimeton and thephorin clearly increased the intensity and duration of adrenaline induced mydriasis in mice.

The effect of chlor-trimeton and thephorin on adrenaline induced hyperglycemia in rats was uncertain and no sure effect could be shown.

In the anaphylactic shock of guinea-pigs the mutually strengthening effect of chlor-trimeton and adrenaline on the one hand and thephorin and adrenaline on the other, seems to be a summation of anti-anaphylactic ability.

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